IN THE SUPREME COURT OF VICTORIA AT MELBOURNE
COMMON LAW DIVISION
JUDICIAL REVIEW AND APPEALS LIST

No. S ECI 2021/03031

B E T W E E N

JONATHAN EDWARD KINGSFORD ANDREWS **Plaintiff**

-and-

PROFESSOR BRETT SUTTON **First Defendant**

(in his capacity as Chief Health Officer
as designated under the *COVID Omnibus (Emergency Measures) Act 2020*)

ASSOCIATE PROFESSOR MICHELLE GILES **Second Defendant**

(in her capacity as Deputy Health Commander
as authorised to exercise emergency powers by
Chief Health Officer under section 199(2)9(a) of
the *Public Health and Wellbeing Act 2008*)

AFFIDAVIT

Date of Document:	24 August 2021	Solicitors Code: 114108	
Filed on behalf of:	Mr Jonathan E. K. Andrews	DX:	
Prepared by:	Mr Jonathan E. K. Andrews	Telephone: (03) 9070 1286	
	Bosanquet Solicitors	Ref:	210801
	TOK Corporate Centre, Level 1 459 Toorak Rd, TOORAK VIC 3142	Email:	info@bosanquets.com

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I, JONATHAN EDWARD KINGSFORD ANDREWS of 1009/1 Almeida Crescent, South Yarra in the State of Victoria, solicitor & barrister, make oath and swear:

1. That I am an Australian citizen being born in Ipswich, Queensland on 21 January 1975, resident and registered voter, in the state of Victoria.

STANDING

2. That as a citizen of Australia and resident of the state of Victoria, I possess constitutional rights under both the State and Commonwealth Constitutions as well as those under common law. Consequently, I also possess legal standing to challenge any unconstitutional, malfeasant, ultra vires and / or invalid actions of the State of Victoria government ("state government") or Federal Government as the case may be. These rights extend to challenge any unlawful, unconstitutional or malfeasant 'ultra vires' actions, that encroach upon my civil liberties and more particularly for the purposes of this suit pertaining to the state government, further explicit rights reserved under the *Charter of Human Rights and Responsibilities 2006*.

STATE GOVERNMENT DECLARATION OF A STATE OF EMERGENCY & STATE OF DISASTER

3. That as a result of the state government's implementation of the *COVID Omnibus (Emergency Measures) Act 2020* ("the COVID Act"), along with its declaration of a state of emergency on 16 March 2020 due to the 'worldwide pandemic',[1] under the *Public Health and Wellbeing Act 2008* ("*Public Health Act*"). The state government concomitantly 'activated' powers therein along with those and that of the *Emergency Management Act 2013* ("the emergency measures"). On 2 August 2020 the state government declared a 'state of disaster' enlarging police powers regarding enforcement of the emergency measures.[2] The state government has thereby invoked emergency legal primacy powers to effectively suspend the State and Commonwealth constitutional rights as well as the Charter rights, whilst these emergency powers remain in place.[3] This has been largely premised upon the result of on the diagnostic method of '*Real time reverse transcription (RT) polymerase chain reaction (PCR) testing for SARS-CoV-2'* ("PCR testing"), in order to determine infectivity rates amongst the Victorian population of 'severe acute respiratory syndrome coronavirus-2' also known as SARS Cov-2 or any other variant thereof such as Delta et al' ("SARS Cov-2").

[1] *Emergency Powers, Public Health and COVID-19 Research Paper No. 2, August 2020,* Mclean and Huf, Department of Parliamentary Services Parliament of Victoria, page 3.
[2] Ibid.
[3] Ibid.

RT-PCR DIAGNOSTIC TESTING

That it is my informed opinion, that in deeply researching this subject matter, that the PCR testing model currently employed *en masse* across Australia is based on the 'Drosten-Corman paper'[4] which at the time of implementation had not been peer reviewed. That since then, credible peer reviewed material pertaining to PCR 'diagnostic' testing also known as the 'Drosten PCR test' annexed hereto and marked as **"JA-01"),** has been discredited by epidemiological and virological experts by '*failing to determine virus infectivity…being poor in detecting replicative virus'*,[5] and '*false positive results are generated by this test, even under controlled laboratory conditions, making it completely unsuitable as a reliable virus screening method*'[6] (peer reviewed material annexed hereto and marked as **"JA-02", "JA-03" & "JA-10").**

WHO DIRECTIVE

That it is my informed opinion, that it is apparent that the Victoria Government has acted upon reports of a 'worldwide pandemic' by the World Health Organisation ("WHO") in declaring the initial state of emergency. It follows that if this is the case then the state government should also be observing the WHO Directive made on 13 January 2020[7] ("WHO Directive") (annexed hereto and marked as **"JA-04"**) advising that in order to prevent false positives from PCR testing, which is the basis for establishing infectivity rates amongst the Victorian population and thereby health directives (such as ongoing arbitrary lockdowns), then these directives ought to be followed in order to prevent erroneous and misleading infectivity rates.

To this end however, the WHO Directive complex and stringent, requiring diligent and focussed clinical reviews with retroactive 'calibrations' of the testing (by personnel

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[4] *Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR* (Corman, Drosten et al), Eurosurveill. 2020;25(3):pii=2000045. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045.

[5] *Predicting Infectious SARS Cov-2 from Diagnostic Samples, Clinical Infectious Diseases, 71(10), 2663-2666* (Bullard, J & Dust, K & Funk et al).

[6] *External peer review of the RTPCR test to detect SARS-CoV-2 reveals 10 major scientific flaws at the molecular and methodological level: consequences for false positive results* 30 November 2020 10.5281 / zenodo.4298004.

[7] *WHO Information Notice for IVD Users 2020/05, Nucleic acid testing (NAT) technologies that use polymerase chain reaction (PCR) for detection of SARS-Cov-2*, 20 January 2021, World Health Organisation.

who are overwhelmingly not qualified general practitioner doctors), being directed to factor in, including but not limited to:

A. the virus load of the sample from the subject;

B. decreased and increased infectivity rates amongst the target population contemporaneous to the taking the samples; and

C. adherence to maximum nucleic acid testing 'cycle thresholds' not being '*inversely proportional to the patient's viral load'* and contemporaneous decreased and increased infectivity rates.

This means that limitations on cycles thresholds during the PCR testing are heavily contingent upon the viral load and infectivity rates at the time of the sample and must be factored in when testing occurs to ward against false positives.

CYCLE THRESHOLDS IN NUCLEIS ACID TESTING

Moreover, Virology experts on PCR testing have advised that significant amounts of 'false positives' are unavoidable when set at *"(greater than) >35 (threshold) cycles only detects signals which do not correlate with infectious virus as determined by isolation in cell culture…if someone is tested by PCR as positive when a threshold of 35 cycles or higher is used (as is the case in most laboratories in Europe & the US), the probability that said person is actually infected is less than 3%, the probability that said result is a false positive is 97%".*[8]

Notwithstanding this, the Victoria Department of Health ("VDoH") states on its webpage entitled '*Assessment and Testing Criteria for coronavirus (COVID-19)*' that:

"This means there is not 'set' Ct (cycle threshold) value to aim for across all platforms. High Ct values are as defined in consultation with the responsible

[8] *External peer review of the RTPCR test to detect SARS-CoV-2 reveals 10 major scientific flaws at the molecular and methodological level: consequences for false positive results* 30 November 2020 10.5281 / zenodo.4298004.

supervising pathologist"[9] (VDoH statement, page 5 annexed hereto and marked as **"JA-05"**).

Therefore, based on this information it appears that the VDoH does not limit the setting cycle thresholds below 35 to militate against false positives, nor is it likely that the VDoH is reasonably following the WHO Directive for calibrating results based on the individual clinical background of the patients and infectivity rates contemporaneous to when the sample was taken (as this is omitted in the VDoH statement). It also appears from the VDoH statement, that individual pathologists are to consult supervising pathologists to determine cycle threshold levels, which does not at all sound 'patient specific' based on individual sample viral loads and individual histories being factored into cycle threshold testing levels if being done 'under supervision'. It is therefore highly doubtful that Victorians are getting competent PCR test results. Furthermore, should the admonitions from leading virologists as to the inherent danger of lackadaisical PCR testing over 35 threshold cycles, we can surmise on the balance of probabilities that false positives have been taken and that lockdowns have been declared on these erroneous bases.

I therefore contend that such directive standards cannot be held as being practically compliable with testing by 'supervised pathologists' on a mass scale, especially with regard to 'drive through' PCR testing sites around Victoria. As it is highly doubtful that such stringent standards have been adhered to regarding PCR testing, it seems an imperative to audit the state governments implementation of these standards admonished under the WHO Directive, in order to ascertain whether the collated PCR testing infectivity rates, used as the bases for public policy including the suspension of constitutional and Charter rights, are or are not, have or have not been accurate and therefore valid.

NOVEL COVID VIRUS

[9] *"The cycle threshold (Ct) value of a reaction is the cycle number when the fluorescence of a PCR product is first detected above the background signal. The lower the Ct value, the more virus is present in the sample being tested, as fewer amplification cycles are required before the threshold for detection is met. A high Ct value generally indicates that it takes longer, that is, more cycles to detect the virus, indication that there is less viral RNA present in the sample. Each PCR assay may have a different Ct value that is used for detecting SARS-CoV-2. Ct values for one in-vitro diagnostic (IVD) device should not be compared with Ct values from other platforms. This means there is no 'set' Ct value to aim for across all platforms. Hight Ct values are as defined in consultation with the responsible supervising pathologist." Assessment and Testing*

The state government appears to be presently relying on 'diagnostic' testing as the scientific basis for acceptance of a SARS-Cov2 as the disease-causing link between symptoms, resulting deaths and the purported 'pandemic', against which it has invoked emergency measures. The Medical Journal of Australia states in its paper entitled *'Isolation and rapid sharing of the 2019 novel coronavirus (SARS CoV-2) from the first patient diagnosed with COVID-19 in Australia'*, that the 'first COVID-19 patient' diagnosed in Australia, was based on the diagnostic method of PCR testing[10] (annexed hereto and marked as "**JA-06**"). This journal article was cited by the Commonwealth Department of Health as alleged proof of 'isolation' of the novel COVID virus (annexed hereto and marked as **"JA-07"**).

4. That it is my informed opinion that RT-PCR and PCR tests are 'diagnostic' only[11] and therefore neither properly isolate nor purify a virus which is only achieved 'via maceration, filtration and use of an ultracentrifuge' ("SOVI isolation"). To this end SOVI isolation (via filtration or purification) is imperative, not only to prove that the subject matter is completely uncontaminated but also that it is also prepared for further animal subject testing of the filtered virus to prove a causative link to death (collectively "hard scientific evidence").[12] This is opposed to PCR testing which adheres to 'particle sequencing' under ultra-sensitive parameters which may be contaminated by dying tissue matter. To this end, the PCR tests only '*look for the 'presence of the COVID virus or of fragments of the COVID virus'*[13] PCR tests therefore can neither be accepted as the best evidence for 'isolation' of the virus, nor definitive proof of a novel SARS-Cov2 being the disease responsible for causing a 'pandemic level death rates'. Short of hard scientific evidence, its basis for the

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Criteria for coronavirus (COVID-19), Victoria Department of Health, Website accessed 18 August 2021, https://www.dhhs.vic.gov.au/assessment-and-testing-criteria-coronavirus-covid-19.

[10] 'Isolation and rapid sharing of the 2019 novel coronavirus (SARS-CoV-2) from the first patient diagnosed with COVID-19 in Australia' The Medical Journal of Australia, Volume 212, Issue 10 page 460, 12 August 2021, https://www.mja.com.au/journal/2020/212/10/isolation-and-rapid-sharing-2019-novel-coronavirus-sars-cov-2-first-patient.

[11] Ibid.

[12] Dr Robert Young MSc DSc PhD, Website accessed 12 August 2021, https://www.drrobertyoung.com/post/the-virus-is-a-concept-that-only-exists-on-a-piece-of-paper

[13] Professor Michael Kidd, Principal Medical Advisor of the Australian Government Department of Health, accessed 12 August 2021, https://www.health.gov.au/resources/videos/top-3-covid-19-vaccine-questions-moderna-vaccine-choice-and-how-tests-differentiate-between-covid-and-flu.

invocation of emergency measures that have suspended constitutional rights *carte blanche*, should be subject to review.

FREEDOM OF INFORMATION DECISIONS ON VIRUS ISOLATION

5. Freedom of Information ("FOI") decision material relevant to this matter from the CSIRO (FOI internal reference no. 2020/50) (annexed hereto and marked as "**JA-08**")[14], and the Commonwealth Department of Health (FOI reference no. 2119). According to these documents, both of these institutions have admitted to being unable to provide clinical proof of SOVI isolated SARS-Cov2 or variant thereof, whereas the 'clinical course' observed by Monash Medical Centre only conducted a RT-PCR test with no causative link studies trials or tests, conducted according to their report (see Annexure JA-06).

6. I therefore respectfully submit that these documents indicate incomplete evidence as to the link between SARS-Cov2 and the disease causing 'pandemic level death rates' and warrant further examination of hard scientific proof of SARS-Cov2 being responsible for COVID-19 deaths under the auspices of this Honourable Court. To this end, a constitutional mandamus is respectfully sought, for the purpose of obtaining 'best evidence' from the Chief Health Officer designated under the COVID Act, namely Professor Brett Sutton and / or the Deputy Health Commander Associate Professor Michelle Giles, within thirty (30) days of the order. That the hard scientific proof be produced to this honourable court, with the overarching purpose of its examination being to remove any doubt as to the validity of the bases state government's declarations.

DAMAGE

9. Furthermore, should the CHO and / or DHC fail to produce the hard scientific proof, it be held that as a result of the invalid invocation of the emergency measures by the state government, it has encroached upon my rights and responsibilities enshrined in the Charter, including but not limited to:

[14]Right to Know Website, Freedom of Information requests, accessed 12 August 2021, https://www.righttoknow.org.au/body/health/unsuccessful?utf8=%E2%9C%93&query=FOI+2119&request_date_after=&request_date_before=&commit=Search#results.

a. Right to protection from torture, cruel and inhumane treatment (s 9);
b. Right to freedom of movement (s 12);
c. Right to peaceful assembly and freedom of association (s 16); and
d. Right to a fair hearing (s 24).

10. That as a result of the application of emergency measures by the state government, it has encroached upon my Federal Constitutional rights, enshrined within the Australian Constitution, including but not limited to:

e. Implied freedom of political association (ss 7 & 24).[15]
f. Express freedom of religion (and congregation) (s 116);
g. Express freedom from prejudice against state of residence (freedom of travel between states) (s 117)[16]; and
h. Implied right to the Rule of Law.[17]

11. That as a result of the foregoing encroachments to my civil liberties by the emergency measures in the COVID Act, I (along with millions of other Victorians), have suffered psychological distress, economic and non-economic damage, limitations to access to justice within the court system.

END TO SPECULATION

13. Finally, that I contend that the purposes of the proposed constitutional mandamus, it will have zero negative impact on the ongoing governance of the state unless the Defendant(s) fails to provide the evidence sought. Moreover, it is clearly in the best interests of the policy makers and its citizenry that the PCR testing be accurate and that speculation and doubt as to the existence or non-existence of the virus attributable to the reported deaths, be ascertained.

[15] *Australian Capital Television Pty Ltd & New South Wales v Commonwealth* [1992] HCA 45; (1992) 177 CLR 106 (30 September 1992).

[16] *Nationwide News Pty Ltd v Wills* [1992] HCA 46.
[17] *Australian Communist Party v The Commonwealth of Australia* (1951).

Either way, ascertaining the vital scientific facts will undoubtedly serve to reinvigorate our collective faith in our state government, its judiciary, our medical apparatus, our liberal democracy and finally our cultural regard for the rule of law.

BIBLIOGRAPHY

14. That may it please the court, I have enclosed an exhaustive list of material relied upon both included and excluded as annexures, which was researched in a humble yet sincere attempt to provide an informed opinion, for the purposes of this Affidavit and the Originating Motion for Judicial Review (Bibliography annexed hereto and marked as **"JA-09").**

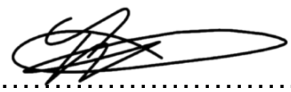
The contents of this affidavit are true and correct and I make it knowing that a person making a false affidavit may be prosecuted for the offences of perjury.

Sworn or Affirmed at SOUTH YARRA
in the State of Victoria on 24 AUGUST 2021

……

The Affidavit & Annexures were sworn to and signed by the Deponent by audio visual link and I, as affidavit taker, have used a scanned or electronic copy of the affidavit & annexures and not the original, in completing the jurat requirements under subsection s 27 ss (1) of the Oaths and Affirmations Act 2018 (VIC).

Before me,



……

Mr Tony Nikolic, Director
Ashley, Francina, Leonard & Associates
Level 25, Tower 3, 300 Barangaroo Avenue,
Sydney, NSW, 2000

(An Australian Legal Practitioner as per Legal Profession Uniform Law [VIC])

on 24 AUGUST 2021

A person authorised under section 19(1) of the **Oaths and Affirmations Act 2018** to take an affidavit.

FORM 43A

Rule 43.06(3)

IN THE SUPREME COURT OF VICTORIA AT MELBOURNE
COMMON LAW DIVISON
JUDICIAL REVIEW AND APPEALS LIST

No. S ECI 2021/03031

B E T W E E N

JONATHAN EDWARD KINGSFORD ANDREWS — Plaintiff

-and-

PROFESSOR BRETT SUTTON
(in his capacity as Chief Health Officer
as designated under the *COVID Omnibus (Emergency Measures) Act 2020*) — First Defendant

ASSOCIATE PROFESSOR MICHELLE GILES
(in her capacity as Deputy Health Commander
as authorised to exercise emergency powers by
Chief Health Officer under section 199(2)9(a) of
the *Public Health and Wellbeing Act 2008*) — Second Defendant

CERTIFICATE IDENTIFYING EXHIBIT

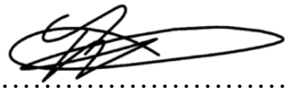
Date of Document:	24 August 2021	Solicitors Code:	141108
Filed on behalf of:	Jonathan E. K. Andrews	DX:	
Prepared by:	Jonathan E. K. Andrews	Telephone:	(03) 9070 1286
	Bosanquet Solicitors,	Ref:	210801
	TOK Corporate Centre, Level 1	Email:	info@bosanquets.com
	459 Toorak Rd, TOORAK VIC 3142		

This is the exhibit marked "**JA-01**" now produced and shown to JONATHAN EDWARD KINGSFORD ANDREWS at the time of swearing that person's affidavit on 24 AUGUST 2021:

.....

JONATHAN EDWARD KINGSFORD ANDREWS

The Affidavit & Annexures were sworn to and signed by the Deponent by audio visual link and I, as affidavit taker, have used a scanned or electronic copy of the affidavit & annexures and not the original, in completing the jurat requirements under subsection s 27 ss (1) of the Oaths and Affirmations Act 2018 (VIC).



……

Mr Tony Nikolic, Director

Ashley, Francina, Leonard & Associates

Level 25, Tower 3, 300 Barangaroo Avenue,

Sydney, NSW, 2000

(An Australian Legal Practitioner as per Legal Profession Uniform Law [VIC])

Exhibit “JA-01”

Drosten PCR test paper.

Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR

Victor M Corman[1], Olfert Landt[2], Marco Kaiser[3], Richard Molenkamp[4], Adam Meijer[5], Daniel KW Chu[6], Tobias Bleicker[1], Sebastian Brünink[1], Julia Schneider[1], Marie Luisa Schmidt[1], Daphne GJC Mulders[4], Bart L Haagmans[4], Bas van der Veer[5], Sharon van den Brink[5], Lisa Wijsman[5], Gabriel Goderski[5], Jean-Louis Romette[7], Joanna Ellis[8], Maria Zambon[8], Malik Peiris[6], Herman Goossens[9], Chantal Reusken[5], Marion PG Koopmans[4], Christian Drosten[1]

1. Charité – Universitätsmedizin Berlin Institute of Virology, Berlin, Germany and German Centre for Infection Research (DZIF), Berlin, Germany
2. Tib-Molbiol, Berlin, Germany
3. GenExpress GmbH, Berlin, Germany*
4. Department of Viroscience, Erasmus MC, Rotterdam, the Netherlands
5. National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands
6. University of Hong Kong, Hong Kong, China
7. Universite d Aix-Marseille, Marseille, France
8. Public Health England, London, United Kingdom
9. Department of Medical Microbiology, Vaccine and Infectious Diseases Institute, University of Antwerp, Antwerp, Belgium

Correspondence: Christian Drosten (christian.drosten@charite.de)

Citation style for this article:
Corman Victor M, Landt Olfert, Kaiser Marco, Molenkamp Richard, Meijer Adam, Chu Daniel KW, Bleicker Tobias, Brünink Sebastian, Schneider Julia, Schmidt Marie Luisa, Mulders Daphne GJC, Haagmans Bart L, van der Veer Bas, van den Brink Sharon, Wijsman Lisa, Goderski Gabriel, Romette Jean-Louis, Ellis Joanna, Zambon Maria, Peiris Malik, Goossens Herman, Reusken Chantal, Koopmans Marion PG, Drosten Christian. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25(3):pii=2000045. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045

Article submitted on 21 Jan 2020 / accepted on 22 Jan 2020 / published on 23 Jan 2020

Background: The ongoing outbreak of the recently emerged novel coronavirus (2019-nCoV) poses a challenge for public health laboratories as virus isolates are unavailable while there is growing evidence that the outbreak is more widespread than initially thought, and international spread through travellers does already occur. **Aim:** We aimed to develop and deploy robust diagnostic methodology for use in public health laboratory settings without having virus material available. **Methods:** Here we present a validated diagnostic workflow for 2019-nCoV, its design relying on close genetic relatedness of 2019-nCoV with SARS coronavirus, making use of synthetic nucleic acid technology. **Results:** The workflow reliably detects 2019-nCoV, and further discriminates 2019-nCoV from SARS-CoV. Through coordination between academic and public laboratories, we confirmed assay exclusivity based on 297 original clinical specimens containing a full spectrum of human respiratory viruses. Control material is made available through European Virus Archive – Global (EVAg), a European Union infrastructure project. **Conclusion:** The present study demonstrates the enormous response capacity achieved through coordination of academic and public laboratories in national and European research networks.

Introduction

According to the World Health Organization (WHO), the WHO China Country Office was informed of cases of pneumonia of unknown aetiology in Wuhan City, Hubei Province, on 31 December 2019 [1]. A novel coronavirus currently termed 2019-nCoV was officially announced as the causative agent by Chinese authorities on 7 January. A viral genome sequence was released for immediate public health support via the community online resource virological.org on 10 January (Wuhan-Hu-1, GenBank accession number MN908947 [2]), followed by four other genomes deposited on 12 January in the viral sequence database curated by the Global Initiative on Sharing All Influenza Data (GISAID). The genome sequences suggest presence of a virus closely related to the members of a viral species termed severe acute respiratory syndrome (SARS)-related CoV, a species defined by the agent of the 2002/03 outbreak of SARS in humans [3,4]. The species also comprises a large number of viruses mostly detected in rhinolophid bats in Asia and Europe.

As at 20 January 2020*, 282 laboratory-confirmed human cases have been notified to WHO [5]. Confirmed cases in travellers from Wuhan were announced on 13 and 17 January in Thailand as well as on 15 January in Japan and 19 January in Korea. The extent of human-to-human transmission of 2019-nCoV is unclear at the time of writing of this report but there is evidence of some human-to-human transmission.

Among the foremost priorities to facilitate public health interventions is reliable laboratory diagnosis. In acute respiratory infection, RT-PCR is routinely used to detect causative viruses from respiratory secretions. We have previously demonstrated the feasibility of introducing robust detection technology based on real-time RT-PCR in public health laboratories during international

Table 1***

Primers and probes, real-time RT-PCR for 2019 novel coronavirus

Assay/use	Oligonucleotide	Sequence[a]	Concentration[b]
RdRP gene	RdRp_SARSr-F	GTGARATGGTCATGTGTGGCGG	Use 600 nM per reaction
	RdRp_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ	Specific for 2019-nCoV, will not detect SARS-CoV. Use 100 nM per reaction and mix with P1
	RdRP_SARSr-P1	FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ	Pan Sarbeco-Probe will detect 2019-nCoV, SARS-CoV and bat-SARS-related CoVs. Use 100 nM per reaction and mix with P2
	RdRp_SARSr-R	CARATGTTAAASACACTATTAGCATA	Use 800 nM per reaction
E gene	E_Sarbeco_F	ACAGGTACGTTAATAGTTAATAGCGT	Use 400 nM per reaction
	E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ	Use 200 nM per reaction
	E_Sarbeco_R	ATATTGCAGCAGTACGCACACA	Use 400 nM per reaction
N gene	N_Sarbeco_F	CACATTGGCACCCGCAATC	Use 600 nM per reaction
	N_Sarbeco_P	FAM-ACTTCCTCAAGGAACAACATTGCCA-BBQ	Use 200 nM per reaction
	N_Sarbeco_R	GAGGAACGAGAAGAGGCTTG	Use 800 nM per reaction

[a] W is A/T; R is G/A; M is A/C; S is G/C. FAM: 6-carboxyfluorescein; BBQ: blackberry quencher.

[b] Optimised concentrations are given in nanomol per litre (nM) based on the final reaction mix, e.g. 1.5 µL of a 10 µM primer stock solution per 25 µL total reaction volume yields a final concentration of 600 nM as indicated in the table.

health emergencies by coordination between public and academic laboratories [6-12]. In all of these situations, virus isolates were available as the primary substrate for establishing and controlling assays and assay performance.

In the present case of 2019-nCoV, virus isolates or samples from infected patients have so far not become available to the international public health community. We report here on the establishment and validation of a diagnostic workflow for 2019-nCoV screening and specific confirmation, designed in absence of available virus isolates or original patient specimens. Design and validation were enabled by the close genetic relatedness to the 2003 SARS-CoV, and aided by the use of synthetic nucleic acid technology.

Methods

Clinical samples and coronavirus cell culture supernatants for initial assay evaluation

Cell culture supernatants containing typed coronaviruses and other respiratory viruses were provided by Charité and University of Hong Kong research laboratories. Respiratory samples were obtained during 2019 from patients hospitalised at Charité medical centre and tested by the NxTAG respiratory pathogen panel (Luminex, S´Hertogenbosch, The Netherlands) or in cases of MERS-CoV by the MERS-CoV upE assay as published before [10]. Additional samples were selected from biobanks at the Rijksinstituut voor Volksgezondheid en Milieu (RIVM), Bilthoven, at Erasmus University Medical Center, Rotterdam, at Public Health England (PHE), London, and at the University of Hong Kong. Samples from all collections comprised sputum as well as nose and throat swabs with or without viral transport medium.

Faecal samples containing bat-derived SARS-related CoV samples (identified by GenBank accession numbers) were tested: KC633203, Betacoronavirus BtCoV/Rhi_eur/BB98–98/BGR/2008; KC633204, Betacoronavirus BtCoV/Rhi_eur/BB98–92/BGR/2008; KC633201, Betacoronavirus BtCoV/Rhi_bla/BB98–22/BGR/2008; GU190221 Betacoronavirus Bat coronavirus BR98–19/BGR/2008; GU190222 Betacoronavirus Bat coronavirus BM98–01/BGR/2008; GU190223, Betacoronavirus Bat coronavirus BM98–13/BGR/2008. All synthetic RNA used in this study was photometrically quantified.

RNA extraction

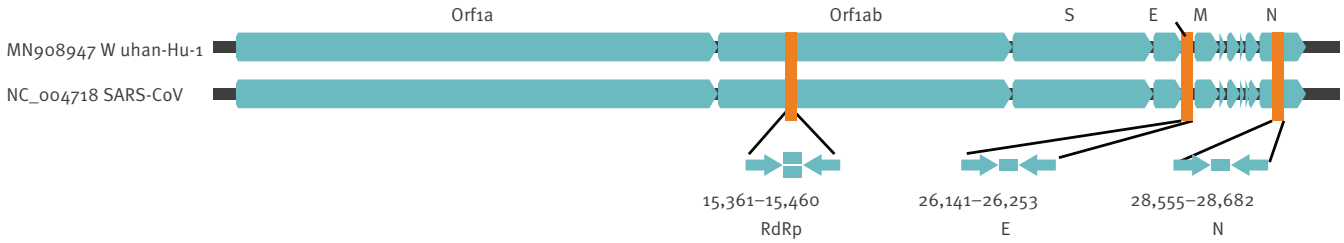
RNA was extracted from clinical samples with the MagNA Pure 96 system (Roche, Penzberg, Germany) and from cell culture supernatants with the viral RNA mini kit (QIAGEN, Hilden, Germany).

Real-time reverse-transcription PCR

A 25 µL reaction contained 5 µL of RNA, 12.5 µL of 2 × reaction buffer provided with the Superscript III one step RT-PCR system with Platinum Taq Polymerase (Invitrogen, Darmstadt, Germany; containing 0.4 mM of each deoxyribont triphosphates (dNTP) and 3.2 mM magnesium sulphate), 1 µL of reverse transcriptase/Taq mixture from the kit, 0.4 µL of a 50 mM magnesium sulphate solution (Invitrogen), and 1 µg of nonacetylated bovine serum albumin (Roche). Primer and probe sequences, as well as optimised concentrations are shown in Table 1. All oligonucleotides were synthesised and provided by Tib-Molbiol (Berlin,

Figure 1

Relative positions of amplicon targets on the SARS coronavirus and the 2019 novel coronavirus genome



E: envelope protein gene; M: membrane protein gene; N: nucleocapsid protein gene; ORF: open reading frame; RdRp: RNA-dependent RNA polymerase gene; S: spike protein gene.

Numbers below amplicons are genome positions according to SARS-CoV, GenBank NC_004718.

Germany). Thermal cycling was performed at 55 °C for 10 min for reverse transcription, followed by 95 °C for 3 min and then 45 cycles of 95 °C for 15 s, 58 °C for 30 s. Participating laboratories used either Roche Light Cycler 480II or Applied Biosystems ViiA7 instruments (Applied Biosystems, Hong Kong, China).

Protocol options and application notes

Laboratories participating in the evaluation used the TaqMan Fast Virus 1-Step Master Mix (Thermo Fisher) with the same oligonucleotide concentrations and cycling conditions. The QIAGEN One-Step RT-PCR Kit was also tested and found to be compatible.

The intended cross-reactivity of all assays with viral RNA of SARS-CoV allows us to use the assays without having to rely on external sources of specific 2019-nCoV RNA.

For a routine workflow, we recommend the E gene assay as the first-line screening tool, followed by confirmatory testing with the RdRp gene assay. Application of the RdRp gene assay with dual colour technology can discriminate 2019-nCoV (both probes positive) from SARS-CoV RNA if the latter is used as positive control. Alternatively, laboratories may choose to run the RdRp assay with only the 2019-nCoV-specific probe.

Ethical statement

The internal use of samples for diagnostic workflow optimisation was agreed under the medical ethical rules of each of the participating partners.

Results

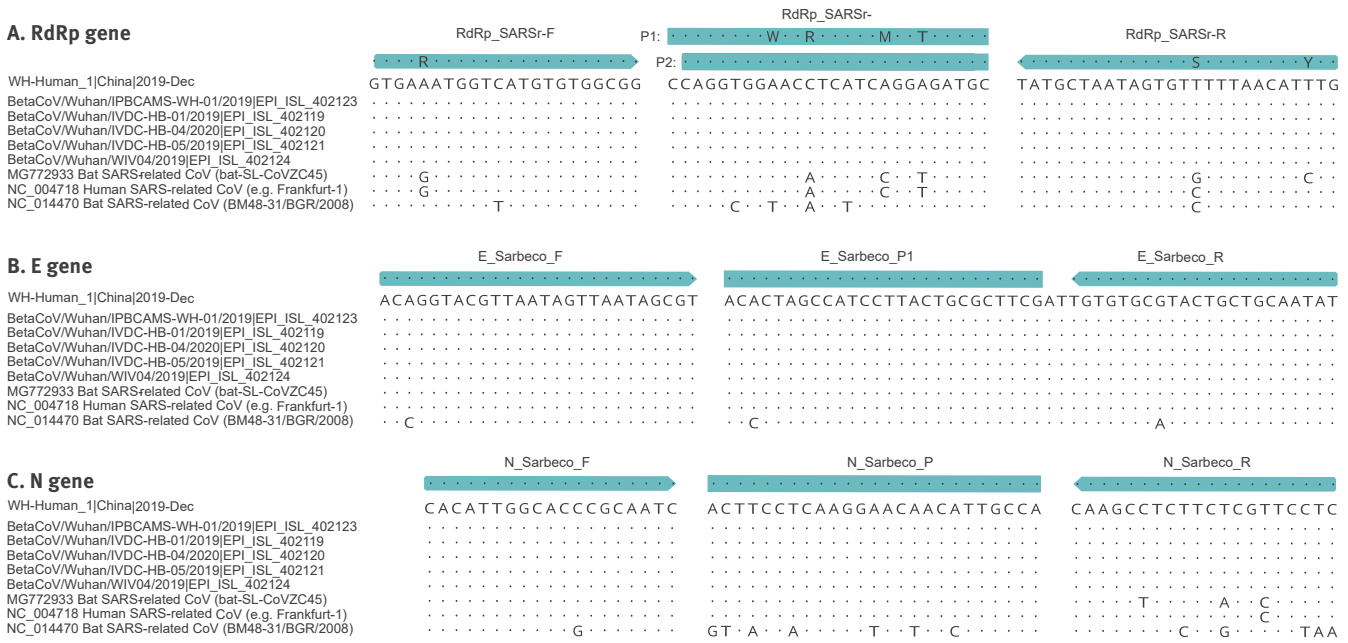
Before public release of virus sequences from cases of 2019-nCoV, we relied on social media reports announcing detection of a SARS-like virus. We thus assumed that a SARS-related CoV is involved in the outbreak. We downloaded all complete and partial (if>400 nt) SARS-related virus sequences available in GenBank by 1 January 2020. The list (n=729 entries) was manually checked and artificial sequences (laboratory-derived, synthetic, etc), as well as sequence duplicates were removed, resulting in a final list of 375 sequences. These sequences were aligned and the alignment was used for assay design (Supplementary Figure S1). Upon release of the first 2019-nCoV sequence at virological.org, three assays were selected based on how well they matched to the 2019-nCoV genome (Figure 1). The alignment was complemented by additional sequences released independently on GISAID (https://www.gisaid.org), confirming the good matching of selected primers to all sequences. Alignments of primer binding domains with 2019-nCoV, SARS-CoV as well as selected bat-associated SARS-related CoV are shown in Figure 2.

Assay sensitivity based on SARS coronavirus virions

To obtain a preliminary assessment of analytical sensitivity, we used purified cell culture supernatant containing SARS-CoV strain Frankfurt-1 virions grown on Vero cells. The supernatant was ultrafiltered and thereby concentrated from a ca 20-fold volume of cell culture supernatant. The concentration step simultaneously reduces the relative concentration of background nucleic acids such as not virion-packaged viral RNA. The virion preparation was quantified by real-time RT-PCR using a specific in vitro-transcribed RNA quantification standard as described in Drosten et al. [8]. All assays were subjected to replicate testing in order to determine stochastic detection frequencies at each assay's sensitivity end point (Figure 3A and B). All assays were highly sensitive, with best results obtained for the E gene and RdRp gene assays (5.2 and 3.8 copies per reaction at 95% detection probability, respectively). These two assays were chosen for further evaluation. One of the laboratories participating in the external evaluation used other basic RT-PCR reagents (TaqMan Fast Virus 1-Step Master Mix) and repeated the sensitivity study, with equivalent results (E gene: 3.2 RNA copies/reaction (95% CI: 2.2–6.8); RdRP: 3.7 RNA copies/reaction (95% CI: 2.8–8.0). Of note, the N gene assay also performed well but was not subjected

Figure 2

Partial alignments of oligonucleotide binding regions, SARS-related coronaviruses (n = 9)



The panels show six available sequences of 2019-nCoV, aligned to the corresponding partial sequences of SARS-CoV strain Frankfurt 1, which can be used as a positive control for all three RT-PCR assays. The alignment also contains a closely related bat virus (Bat SARS-related CoV isolate bat-SL-CoVZC45, GenBank accession number MG772933) as well as the most distant member within the SARS-related bat CoV clade, detected in Bulgaria (GenBank accession number NC_014470). Dots represent identical nucleotides compared with the WH_Human_1 sequence. Nucleotide substitutions are specified. Blue arrows: oligonucleotides as specified in Table 1. More comprehensive alignments can be found in the Supplement.

to intensive further validation because it was slightly less sensitive (Supplementary Figure S2)

Sensitivity based on in vitro-transcribed RNA identical to 2019 novel coronavirus target sequences

Although both assays detected 2019-nCoV without polymorphisms at oligonucleotide binding sites (Figure 2), we additionally generated in vitro-transcribed RNA standards that exactly matched the sequence of 2019-nCoV for absolute quantification and studying the limit of detection (LOD). Replicate reactions were done at concentrations around the detection end point determined in preliminary dilution experiments. The resulting LOD from replicate tests was 3.9 copies per reaction for the E gene assay and 3.6 copies per reaction for the RdRp assay (Figure 3C and D). These figures were close to the 95% hit rate of 2.9 copies per reaction, according to the Poisson distribution, expected when one RNA molecule is detected.

Discrimination of 2019 novel coronavirus from SARS coronavirus by RdRp assay

Following the rationale that SARS-CoV RNA can be used as a positive control for the entire laboratory procedure, thus obviating the need to handle 2019-nCoV RNA, we formulated the RdRp assay so that it contains two probes: a broad-range probe reacting with SARS-CoV and 2019-nCoV and an additional probe that reacts only with 2019-nCoV. By limiting dilution experiments, we confirmed that both probes, whether used individually or in combination, provided the same LOD for each target virus. The specific probe RdRP_SARSr-P2 detected only the 2019-nCoV RNA transcript but not the SARS-CoV RNA.

Detection range for SARS-related coronaviruses from bats

At present, the potential exposure to a common environmental source in early reported cases implicates the possibility of independent zoonotic infections with increased sequence variability [5]. To show that the assays can detect other bat-associated SARS-related viruses, we used the E gene assay to test six bat-derived faecal samples available from Drexler et al. [13] und Muth et al. [14]. These virus-positive samples stemmed from European rhinolophid bats. Detection of these phylogenetic outliers within the SARS-related CoV clade suggests that all Asian viruses are likely to be detected. This would, theoretically, ensure broad sensitivity even in case of multiple independent acquisitions of variant viruses from an animal reservoir.

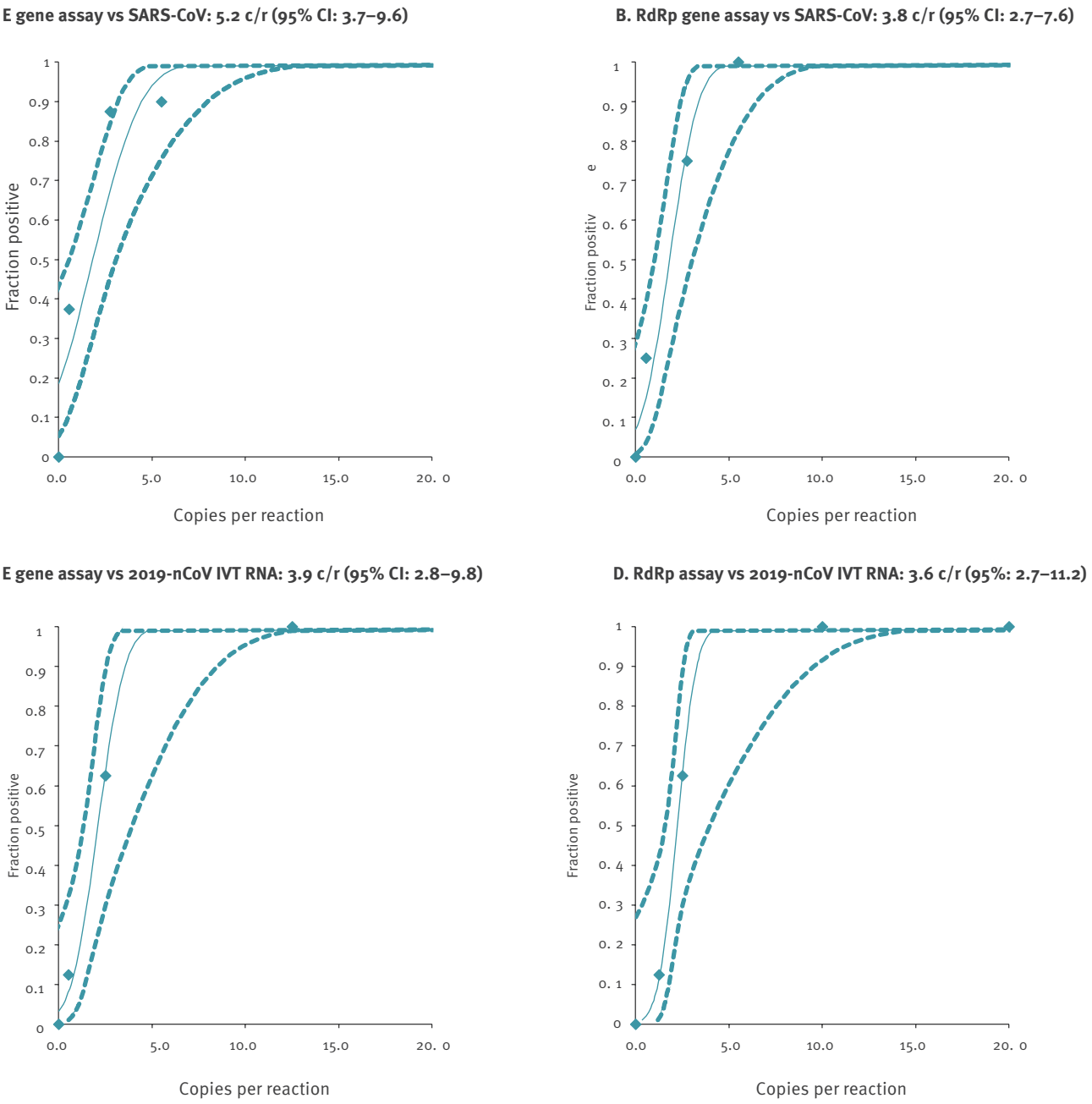
Specificity testing

Chemical stability

To exclude non-specific reactivity of oligonucleotides among each other, causing artificial fluorescent

Figure 3

Determination of limits of detection based on SARS coronavirus genomic RNA and 2019 novel coronavirus-specific in vitro transcribed RNA



CI: confidence intervals; c/r: copies per reaction; IVT: in vitro-transcribed RNA.

A: E gene assay, evaluated with SARS-CoV genomic RNA. B: RdRp gene assay evaluated with SARS-CoV genomic RNA. C: E-gene assay, evaluated with 2019-nCoV-specific in vitro-transcribed RNA standard. D: RdRp gene assay evaluated with 2019-nCoV-specific in vitro-transcribed RNA standard.

The x-axis shows input RNA copies per reaction. The y-axis shows positive results in all parallel reactions performed, squares are experimental data points resulting from replicate testing of given concentrations (x-axis) in parallels assays (eight replicate reactions per point).

Technical limits of detection are given in the panels headings. The inner line is a probit curve (dose-response rule). The outer dotted lines are 95% CI.

Table 2

Tests of known respiratory viruses and bacteria in clinical samples and cell culture preparations for cross-reactivity in 2019 novel coronavirus E and RdRp gene assays (n = 310)

Clinical samples with known viruses	Clinical samples[a]	Virus isolates[b]
HCoV-HKU1	14	1[c]
HCoV-OC43	16	2[d]
HCoV-NL63	14	1[e]
HCoV-229E	18	2[f]
MERS-CoV	5	1[g]
Influenza A(H1N1)pdm09	17	1
Influenza A(H3N2)	16	1
Influenza A (untyped)	11	NA
Influenza A(H5N1)	1	1
Influenza A(H7N9)	0	1
Influenza B (Victoria or Yamagata)	31	1
Rhinovirus/enterovirus	31	NA
Respiratory syncytial virus (A/B)	33	NA
Parainfluenza 1 virus	12	NA
Parainfluenza 2 virus	11	NA
Parainfluenza 3 virus	14	NA
Parainfluenza 4 virus	11	NA
Human metapneumovirus	16	NA
Adenovirus	13	1
Human bocavirus	6	NA
Legionella spp.	3	NA
Mycoplasma spp.	4	NA
Total clinical samples	**297**	**NA**

[a] For samples with multiple viruses detected, the virus with highest concentration is listed, as indicated by real-time PCR Ct value.

[b] Directly quantified or spiked in human negative-testing sputum.

[c] 1×10^5 RNA copies/mL, determined by specific real-time RT-PCR. Isolated from human airway epithelial culture.

[d] 1×10^{10} RNA copies/mL, determined by specific real-time RT-PCR of one isolate. The other isolate was not quantified but spiked in human negative-testing sputum.

[e] 4×10^9 RNA copies/mL, determined by specific real-time RT-PCR.

[f] 3×10^9 RNA copies/mL, determined by specific real-time RT-PCR of one isolate. The other isolate was not quantified spiked in human negative-testing sputum.

[g] 1×10^8 RNA copies/mL, determined by specific real-time RT-PCR.

signals, all assays were tested 120 times in parallel with water and no other nucleic acid except the provided oligonucleotides. In none of these reactions was any positive signal detected.

Cross-reactivity with other coronaviruses

Cell culture supernatants containing all endemic human coronaviruses (HCoV)229E, NL63, OC43 and HKU1 as well as MERS-CoV were tested in duplicate in all three assays (Table 2). For the non-cultivable HCoV-HKU1, supernatant from human airway culture was used. Viral RNA concentration in all samples was determined by specific real-time RT-PCRs and in vitro-transcribed RNA standards designed for absolute quantification of viral load. Additional undiluted (but not quantified) cell culture supernatants were tested as summarised in Table 2. These were additionally mixed into negative human sputum samples. None of the tested viruses or virus preparations showed reactivity with any assay.

Exclusivity of 2019 novel coronavirus based on clinical samples pre-tested positive for other respiratory viruses

Using the E and RdRp gene assays, we tested a total of 297 clinical samples from patients with respiratory disease from the biobanks of five laboratories that provide diagnostic services (one in Germany, two in the Netherlands, one in Hong Kong, one in the UK). We selected 198 samples from three university medical centres where patients from general and intensive care wards as well as mainly paediatric outpatient departments are seen (Germany, the Netherlands, Hong Kong). The remaining samples were contributed by national public health services performing surveillance studies (RIVM, PHE), with samples mainly submitted by practitioners. The samples contained the broadest range of respiratory agents possible and reflected the general spectrum of virus concentrations encountered in diagnostic laboratories in these countries (Table 2). In total, this testing yielded no false positive outcomes. In four individual test reactions, weak initial reactivity was seen but they were negative upon retesting with the same assay. These signals were not associated with any particular virus, and for each virus with which initial positive reactivity occurred, there were other samples that contained the same virus at a higher concentration but did not test positive. Given the results from the extensive technical qualification described above, it was concluded that this initial reactivity was not due to chemical instability of real-time PCR probes but most probably to handling issues caused by the rapid introduction of new diagnostic tests and controls during this evaluation study.

Discussion

The present report describes the establishment of a diagnostic workflow for detection of an emerging virus in the absence of physical sources of viral genomic nucleic acid. Effective assay design was enabled by the willingness of scientists from China to share genome information before formal publication, as well as the availability of broad sequence knowledge from ca 15 years of investigation of SARS-related viruses in animal reservoirs. The relative ease with which assays could be designed for this virus, in contrast to SARS-CoV in 2003, proves the huge collective value of descriptive studies of disease ecology and viral genome diversity [8,15-17].

Real-time RT-PCR is widely deployed in diagnostic virology. In the case of a public health emergency, proficient diagnostic laboratories can rely on this robust technology to establish new diagnostic tests within their routine services before pre-formulated assays become available. In addition to information on

reagents, oligonucleotides and positive controls, laboratories working under quality control programmes need to rely on documentation of technical qualification of the assay formulation as well as data from external clinical evaluation tests. The provision of control RNA templates has been effectively implemented by the EVAg project that provides virus-related reagents from academic research collections [18]. SARS-CoV RNA was retrievable from EVAg before the present outbreak; specific products such as RNA transcripts for the here-described assays were first retrievable from the EVAg online catalogue on 14 January 2020 (https://www.european-virus-archive.com). Technical qualification data based on cell culture materials and synthetic constructs, as well as results from exclusivity testing on 75 clinical samples, were included in the first version of the diagnostic protocol provided to the WHO on 13 January 2020. Based on efficient collaboration in an informal network of laboratories, these data were augmented within 1 week comprise testing results based on a wide range of respiratory pathogens in clinical samples from natural infections. Comparable evaluation studies during regulatory qualification of in vitro diagnostic assays can take months for organisation, legal implementation and logistics and typically come after the peak of an outbreak has waned. The speed and effectiveness of the present deployment and evaluation effort were enabled by national and European research networks established in response to international health crises in recent years, demonstrating the enormous response capacity that can be released through coordinated action of academic and public laboratories [18-22]. This laboratory capacity not only supports immediate public health interventions but enables sites to enrol patients during rapid clinical research responses.

*Author's correction

The sentence As at 20 January 2020, 282 laboratory-confirmed human cases have been notified to WHO was originally published with a wrong date (As at 20 January 2019...). This mistake was corrected on 8 April 2020.

On 29 July 2020 the correct affiliation of Marco Kaiser was added and the remaining affiliations were renumbered.

**Addendum

The Conflict of interest section was updated on 29 July 2020.

***Erratum

In the second half of Table 1, nM (nanomolar) was misspelled as nm when this article was published. This mistake was corrected on 4 February 2021. We apologise for any inconvenience this typo may have caused.

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We gratefully acknowledge the authors, the originating and submitting laboratories for their sequence and metadata shared through GISAID, on which this research is based. All authors of data may be contacted directly via www.gisaid.org: National Institute for Viral Disease Control and Prevention, China CDC (Wenjie Tan, Xiang Zhao, Wenling Wang, Xuejun Ma, Yongzhong Jiang, Roujian Lu, Ji Wang, Weimin Zhou, Peihua Niu, Peipei Liu,Faxian Zhan, Weifeng Shi, Baoying Huang, Jun Liu, Li Zhao, Yao Meng, Xiaozhou He, Fei Ye, Na Zhu, Yang Li, Jing Chen, Wenbo Xu, George F. Gao, Guizhen Wu); Wuhan Institute of Virology, Chinese Academy of Sciences (Peng Zhou, Xing-Lou Yang, Ding-Yu Zhang, Lei Zhang, Yan Zhu, Hao-Rui Si, Zhengli Shi); Institute of Pathogen Biology, Chinese Academy of Medical Sciences and Peking Union Medical College (Lili Ren, Jianwei Wang, Qi Jin, Zichun Xiang, Yongjun Li, Zhiqiang Wu, Chao Wu, Yiwei Liu); and National Institute for Communicable Disease Control and Prevention (ICDC), China CDC (Zhang Y-Z, Wu, F, Chen Y-M, Pei Y-Y, Xu L, Wang W, Zhao S, Yu B, Hu Y, Tao Z-W, Song Z-G, Tian J-H, Zhang Y-L, Liu Y, Zheng J-J, Dai F-H, Wang Q-M, She J-L and Zhu T-Y)

We thank Marta Zuchowski, Sigrid Kersten, and Joerg Hofmann for help with sample logistics. In vitro-transcribed control RNA for the E gene assay can be acquired from author C. D. through the European Virus Archive platform (www.european-virus-archive.com),

Conflict of interest **

Olfert Landt is CEO of Tib-Molbiol; Marco Kaiser is senior researcher at GenExpress and serves as scientific advisor for Tib-Molbiol.

Authors' contributions

VMC: Planned and conducted experiments, conceptualised the laboratory work

OL: Planned and conducted experiments, conceptualised the laboratory work

MK: Planned and conducted experiments

RM: Planned and conducted experiments, conceptualised the laboratory work

AM: Planned and conducted experiments, conceptualised the laboratory work

DKWC: Planned and conducted experiments

TB: Planned and conducted experiments

SB: Planned and conducted experiments

JS: Planned and conducted experiments

MLS: Planned and conducted experiments

DGJCM: Planned and conducted experiments

BLH: Planned and conducted experiments

BvdV: Planned and conducted experiments

SvdB: Planned and conducted experiments

LW: Planned and conducted experiments

GG: Planned and conducted experiments

JLR: Contributed to overall study conceptualization

JE: Planned and conducted experiments, conceptualised the laboratory work

MZ: Planned laboratory work, contributed to overall study conceptualization

MP: Planned laboratory work, contributed to overall study conceptualization

HG: Contributed to overall study conceptualization

CR: Planned experiments, conceptualised the laboratory work

MPGK: Planned experiments, conceptualised the laboratory work

CD: Planned experiments, conceptualised the laboratory work, conceptualised the overall study, wrote the manuscript draft.

References

1. World Health Organization (WHO). Coronavirus. Geneva: WHO; 2020 [Accessed 21 Jan 2020]. Available from: https://www.who.int/health-topics/coronavirus
2. Zhang Y-Z. Novel 2019 coronavirus genome. Virological. [Accessed 21 Jan 2020]. Available from: http://virological.org/t/novel-2019-coronavirus-genome/319
3. de Groot RJ, Baker SC, Baric R, Enjuanes L, Gorbalenya AE, Holmes KV, et al. Family Coronaviridae. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ. Virus taxonomy: classification and nomenclature of viruses: ninth report of the International Committee on Taxonomy of Viruses. London; Waltham: Academic Press; 2012. p. 806-20.
4. Peiris JS, Yuen KY, Osterhaus AD, Stöhr K. The severe acute respiratory syndrome. N Engl J Med. 2003;349(25):2431-41. https://doi.org/10.1056/NEJMra032498 PMID: 14681510
5. World Health Organization. (WHO. Novel Coronavirus (2019-nCoV). Situation report – 1. Geneva: WHO; 21 Jan 2020. Available from: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200121-sitrep-1-2019-ncov.pdf
6. Abbott A. SARS testing: First past the post. Nature. 2003;423(6936):114. https://doi.org/10.1038/423114a PMID: 12736651
7. Corman VM, Müller MA, Costabel U, Timm J, Binger T, Meyer B, et al. Assays for laboratory confirmation of novel human coronavirus (hCoV-EMC) infections. Euro Surveill. 2012;17(49):20334. https://doi.org/10.2807/ese.17.49.20334-en PMID: 23231891
8. Drosten C, Günther S, Preiser W, van der Werf S, Brodt HR, Becker S, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med. 2003;348(20):1967-76. https://doi.org/10.1056/NEJMoa030747 PMID: 12690091
9. Corman VM, Eickmann M, Landt O, Bleicker T, Brünink S, Eschbach-Bludau M, et al. Specific detection by real-time reverse-transcription PCR assays of a novel avian influenza A(H7N9) strain associated with human spillover infections in China. Euro Surveill. 2013;18(16):20461. PMID: 23611031
10. Corman VM, Eckerle I, Bleicker T, Zaki A, Landt O, Eschbach-Bludau M, et al. Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. Euro Surveill. 2012;17(39):20285. https://doi.org/10.2807/ese.17.39.20285-en PMID: 23041020
11. Panning M, Charrel RN, Donoso Mantke O, Landt O, Niedrig M, Drosten C. Coordinated implementation of chikungunya virus reverse transcription-PCR. Emerg Infect Dis. 2009;15(3):469-71. https://doi.org/10.3201/eid1503.081104 PMID: 19239767
12. Corman VM, Rasche A, Baronti C, Aldabbagh S, Cadar D, Reusken CB, et al. Assay optimization for molecular detection of Zika virus. Bull World Health Organ. 2016;94(12):880-92. https://doi.org/10.2471/BLT.16.175950 PMID: 27994281
13. Drexler JF, Gloza-Rausch F, Glende J, Corman VM, Muth D, Goettsche M, et al. Genomic characterization of severe acute respiratory syndrome-related coronavirus in European bats and classification of coronaviruses based on partial RNA-dependent RNA polymerase gene sequences. J Virol. 2010;84(21):11336-49. https://doi.org/10.1128/JVI.00650-10 PMID: 20686038
14. Muth D, Corman VM, Roth H, Binger T, Dijkman R, Gottula LT, et al. Attenuation of replication by a 29 nucleotide deletion in SARS-coronavirus acquired during the early stages of human-to-human transmission. Sci Rep. 2018;8(1):15177. https://doi.org/10.1038/s41598-018-33487-8 PMID: 30310104
15. Corman VM, Muth D, Niemeyer D, Drosten C. Hosts and sources of endemic human coronaviruses. Adv Virus Res. 2018;100:163-88. https://doi.org/10.1016/bs.aivir.2018.01.001 PMID: 29551135
16. Drexler JF, Corman VM, Drosten C. Ecology, evolution and classification of bat coronaviruses in the aftermath of SARS. Antiviral Res. 2014;101:45-56. https://doi.org/10.1016/j.antiviral.2013.10.013 PMID: 24184128
17. Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol. 2019;17(3):181-92. https://doi.org/10.1038/s41579-018-0118-9 PMID: 30531947
18. Romette JL, Prat CM, Gould EA, de Lamballerie X, Charrel R, Coutard B, et al. The European Virus Archive goes global: A growing resource for research. Antiviral Res. 2018;158:127-34. https://doi.org/10.1016/j.antiviral.2018.07.017 PMID: 30059721
19. Alleweldt F, Kara S, Osinski A, Van Baal P, Kellerborg K, Aarestrup FM, et al. Developing a framework to assess the costeffectiveness of COMPARE - a global platform for the exchange of sequence-based pathogen data. Rev Sci Tech. 2017;36(1):311-22. https://doi.org/10.20506/rst.36.1.2631 PMID: 28926006
20. Domingo C, Ellerbrok H, Koopmans M, Nitsche A, Leitmeyer K, Charrel RN, et al. Need for additional capacity and improved capability for molecular detection of yellow fever virus in European Expert Laboratories: External Quality Assessment, March 2018. Euro Surveill. 2018;23(28):1800341. https://doi.org/10.2807/1560-7917.ES.2018.23.28.1800341 PMID: 30017021
21. Pas SD, Patel P, Reusken C, Domingo C, Corman VM, Drosten C, et al. First international external quality assessment of molecular diagnostics for Mers-CoV. J Clin Virol. 2015;69:81-5. https://doi.org/10.1016/j.jcv.2015.05.022 PMID: 26209385
22. Gobat N, Amuasi J, Yazdanpanah Y, Sigfid L, Davies H, Byrne JP, et al. Advancing preparedness for clinical research during infectious disease epidemics. ERJ Open Res. 2019;5(2):00227-2018. https://doi.org/10.1183/23120541.00227-2018 PMID: 31123684

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FORM 43A

Rule 43.06(3)

IN THE SUPREME COURT OF VICTORIA AT MELBOURNE
COMMON LAW DIVISON
JUDICIAL REVIEW AND APPEALS LIST

No. S ECI 2021/03031

B E T W E E N

JONATHAN EDWARD KINGSFORD ANDREWS — Plaintiff

-and-

PROFESSOR BRETT SUTTON
(in his capacity as Chief Health Officer
as designated under the *COVID Omnibus (Emergency Measures) Act 2020*) — First Defendant

ASSOCIATE PROFESSOR MICHELLE GILES
(in her capacity as Deputy Health Commander
as authorised to exercise emergency powers by
Chief Health Officer under section 199(2)9(a) of
the *Public Health and Wellbeing Act 2008*) — Second Defendant

CERTIFICATE IDENTIFYING EXHIBIT

Date of Document:	24 August 2021	Solicitors Code:	141108
Filed on behalf of:	Jonathan E. K. Andrews	DX:	
Prepared by:	Jonathan E. K. Andrews	Telephone:	(03) 9070 1286
	Bosanquet Solicitors,	Ref:	210801
	TOK Corporate Centre, Level 1	Email:	info@bosanquets.com
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This is the exhibit marked "**JA-02**" now produced and shown to JONATHAN EDWARD KINGSFORD ANDREWS at the time of swearing/affirming that person's affidavit on 24 AUGUST 2021:

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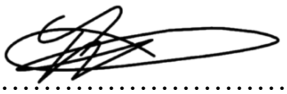
JONATHAN EDWARD KINGSFORD ANDREWS

The Affidavit & Annexures were sworn to and signed by the Deponent by audio visual link and I, as affidavit taker, have used a scanned or electronic copy of the affidavit & annexures and not the original, in completing the jurat requirements under subsection s 27 ss (1) of the Oaths and Affirmations Act 2018 (VIC).



……

Mr Tony Nikolic, Director

Ashley, Francina, Leonard & Associates

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Sydney, NSW, 2000

(An Australian Legal Practitioner as per Legal Profession Uniform Law [VIC])

Exhibit “JA-02”

Predicting Infectious SARS Cov-2 from Diagnostic Samples.

Clinical Infectious Diseases

MAJOR ARTICLE

Predicting Infectious Severe Acute Respiratory Syndrome Coronavirus 2 From Diagnostic Samples

Jared Bullard,[1,2,3] Kerry Dust,[1] Duane Funk,[4,5] James E. Strong,[2,3,4] David Alexander,[1,3] Lauren Garnett,[3,4] Carl Boodman,[3] Alexander Bello,[3,4] Adam Hedley,[1] Zachary Schiffman,[3,4] Kaylie Doan,[4] Nathalie Bastien,[3,4] Yan Li,[3,4] Paul G. Van Caeseele,[1,2,3] and Guillaume Poliquin[2,3,4]

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(See the Editorial Commentary by Binnicker on pages 2667–8.)

Background. Reverse-transcription polymerase chain reaction (RT-PCR) has become the primary method to diagnose viral diseases, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). RT-PCR detects RNA, not infectious virus; thus, its ability to determine duration of infectivity of patients is limited. Infectivity is a critical determinant in informing public health guidelines/interventions. Our goal was to determine the relationship between E gene SARS-CoV-2 RT-PCR cycle threshold (Ct) values from respiratory samples, symptom onset to test (STT), and infectivity in cell culture.

Methods. In this retrospective cross-sectional study, we took SARS-CoV-2 RT-PCR–confirmed positive samples and determined their ability to infect Vero cell lines.

Results. Ninety RT-PCR SARS-CoV-2–positive samples were incubated on Vero cells. Twenty-six samples (28.9%) demonstrated viral growth. Median tissue culture infectious dose/mL was 1780 (interquartile range, 282–8511). There was no growth in samples with a Ct > 24 or STT > 8 days. Multivariate logistic regression using positive viral culture as a binary predictor variable, STT, and Ct demonstrated an odds ratio (OR) for positive viral culture of 0.64 (95% confidence interval [CI], .49–.84; $P < .001$) for every 1-unit increase in Ct. Area under the receiver operating characteristic curve for Ct vs positive culture was OR, 0.91 (95% CI, .85–.97; $P < .001$), with 97% specificity obtained at a Ct of > 24.

Conclusions. SARS-CoV-2 Vero cell infectivity was only observed for RT-PCR Ct < 24 and STT < 8 days. Infectivity of patients with Ct > 24 and duration of symptoms > 8 days may be low. This information can inform public health policy and guide clinical, infection control, and occupational health decisions. Further studies of larger size are needed.

Keywords. SARS-CoV-2; COVID-19; RT-PCR; infectivity; public health.

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), represents a public health emergency of historic proportion. The global containment efforts have had broad societal and economic impacts. Policy decisions to relax public health measures will require a better understanding of duration of infectivity. This information will also impact infection control practices and occupational health.

To date, the diagnosis of COVID-19 has relied on the detection of SARS-CoV-2 through molecular detection. While this method is both rapid and highly sensitive, there are important limitations. Several studies describe the persistence of SARS-CoV-2 RNA within different body sites [1, 2]. It is known from other viruses that viral RNA can persist beyond infectivity [3, 4]. As a result, demonstration of in vitro infectiousness on cell lines is a more informative surrogate of viral transmission. The ability of viral culture to inform infectivity is an important aspect of diagnostics, but its use is hampered by its difficult and labor-intensive nature. This is further complicated by the need for Biosafety Level 3 facilities in the case of SARS-CoV-2. In a recent cohort study of 9 patients, no virus could be recovered beyond 7 days after symptom onset [1]. This important study is limited by the small number of patients examined and the fact that all 9 cases are linked; therefore, the data may represent a unique viral subpopulation. Here we add to the existing body of literature by presenting viral culture results on a larger cross-sectional group of patients, compared to polymerase chain reaction (PCR) data and time of symptom onset.

MATERIALS AND METHODS

SARS-CoV-2 Reverse-Transcription PCR Cycle Threshold Values and Symptom Onset to Test

All samples in this study were obtained to support routine care and surveillance of the public health response in the province of Manitoba, Canada. All suspected COVID-19 cases had

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SARS-CoV-2 reverse-transcription PCR (RT-PCR) performed on nasopharyngeal (NP) or endotracheal (ETT) samples at Cadham Provincial Laboratory (CPL), the public health laboratory.

The NP swabs and ETT specimens in viral transport media were stored at 4°C for 24–72 hours until they were tested for the presence of SARS-CoV-2 RNA using real-time RT-PCR targeting a 122-nt portion of the Sarbecovirus envelope gene (E gene) [5]. Fifty-five microliters of RNA was extracted from 200 µL of a respiratory specimen using the Ambion AM1836 RNA kit (Thermo Fisher) paired with the Kingfisher Flex instrument (Thermo Fisher). The 20 µL reactions, comprised of TaqMan Fast Virus One-step master mix and 5 µL of RNA, were cycled for 5 minutes at 50°C, 20 seconds at 95°C followed by 40 cycles of 5 seconds at 95°C and 30 seconds at 58°C on a Bio-Rad CFX96 thermal cycler. RT-PCR results were analyzed with the CFX manager software (version 3.1).

Through public health and epidemiology/surveillance and laboratory records, date of symptom onset was determined. Time from symptom onset to RT-PCR, or symptoms to test (STT), was calculated based on laboratory records. For all positive samples, the cycle threshold (Ct) was obtained. The study was performed in accordance with protocol HS23906 (H2020:211), approved by the University of Manitoba Research Ethics Board.

Median Tissue Culture Infectious Dose Assay

Samples were stored at −80°C for between 2 and 4 weeks before being processed for culture. Viral titers of patient samples were determined through median tissue culture infectious dose ($TCID_{50}$) assays inside a Biosafety Level 4 laboratory. In brief, Vero cells (ATCC: CCL-81), maintained in modified Eagle's medium (MEM) supplemented with 5% fetal bovine serum (FBS), 1% penicillin/streptomycin, 0.5 µg/mL amphotericin B, and 1% L-glutamine, were seeded into 96-well plates (Thermo Scientific, 167008) at 70% confluency. Using dilution blocks, patient samples were serially diluted 10-fold from 10^{-1} to 10^{-8} in MEM supplemented with 2% FBS, 1% penicillin/streptomycin, 0.5 µg/mL amphotericin B, and 1% L-glutamine. Dilutions were placed onto the Vero cells in triplicate and incubated at 37°C with 5% carbon dioxide for 96 hours. Following incubation of 4 days, cytopathic effect was evaluated under a microscope and recorded. $TCID_{50}$ and $TCID_{50}$/mL were calculated using the Reed and Muench method previously described [6]

Statistical Methods

Data are presented as mean ± standard deviation for normally distributed data and as median with interquartile range (IQR) for nonnormally distributed data. *P* values are reported as 2-tailed. All statistical analysis was performed with Stata version 14.2 (StataCorp, College Station, Texas). Between-group comparisons were performed using a Student *t* test or Mann-Whitney test. Normality was assessed using the Kolmogorov-Smirnov test, and logistic regression was performed with robust standard errors.

RESULTS

A total of 90 samples were analyzed. Median age of the patients sampled was 45 (IQR, 30–59) years. Almost half (49%) of our samples were from males. SARS-CoV-2 was successfully cultivated from 26 (28.9%) of the samples. The samples included in this study included those positive for SARS-CoV-2 by RT-PCR from day of symptom onset (day 0) up to 21 days after symptom onset. Within this range of samples, positive cultures were only observed up to day 8 after symptom onset (Figure 1). Median Ct count of all samples was 23 (IQR, 17–32). The median $TCID_{50}$/mL was 1780 (IQR, 282–8511). Positive culture samples had a significantly lower Ct compared with culture-negative samples (17 [IQR, 16–18] vs 27 [IQR, 22–33]; $P < .001$; Figure 2). Symptom to test time was also significantly lower in culture-positive vs culture-negative samples (3 [IQR, 2–4] days vs 7 [IQR, 4–11] days; $P < .001$; Figure 2).

Multivariate logistic regression using positive culture as a predictor variable (binary result) and STT, age, and sex as independent variables showed Ct as being significant (odds ratio [OR], 0.64 [95% confidence interval {CI}, .49–.84]; $P < .001$). This implies that for every 1-unit increase in Ct, the odds of a positive culture decreased by 32%. Increasing symptom to test time was also significantly associated with a negative culture (OR, 0.63 [95% CI, .42–.94]; $P = .025$). For every 1-day increase in STT, the odds ratio of being culture positive was decreased by 37%. Receiver operating characteristic curves constructed using Ct vs positive culture showed an area of 0.91 (95% CI, .85–.97; $P < .001$) with 97% specificity obtained at a Ct of > 24. Similarly, STT vs positive culture showed an area of 0.81 (95% CI .73–.90; $P < .001$), with 96% specificity at > 8 days. The probability of successfully cultivating SARS-CoV-2 on Vero cell culture compared to STT is demonstrated in Figure 3. The probability of

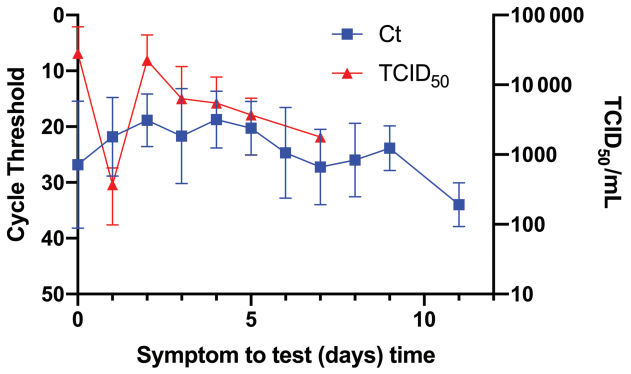

Figure 1. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral dynamics as expressed by E gene reverse-transcription polymerase chain reaction cycle threshold (Ct) value and cell culture median tissue culture infectious dose ($TCID_{50}$)/mL, over time (days).

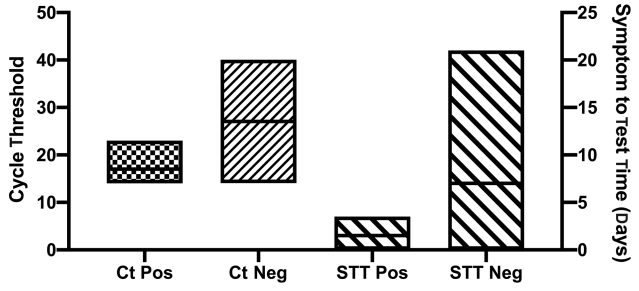

Figure 2. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) E gene reverse-transcription polymerase chain reaction cycle threshold (Ct) values and symptom to test time (STT) in samples that were culture positive or negative. Positive SARS-CoV-2 culture samples had a significantly lower Ct compared with culture-negative samples (17 [interquartile range {IQR}, 16–18] vs 27 [IQR, 22–33]; $P < .001$). STT was also significantly lower in culture-positive vs culture-negative samples (3 [IQR, 2–4] days vs 7 [IQR, 4–11] days; $P < .001$).

obtaining a positive viral culture peaked on day 3 and decreased from that point.

DISCUSSION

PCR and other nucleic amplification (NA) strategies have surpassed viral culture as the gold standard viral diagnostic, because of their wider application, higher sensitivity, rapid performance, and ability for field deployment. A major drawback to PCR and other diagnostic approaches (including other NA, serology, and antigen detection) is that they all fail to determine virus infectivity; PCR sensitivity is excellent but specificity for detecting replicative virus is poor [7]. Our study utilized a cross-sectional approach to correlate COVID-19 symptom onset to specimen collection with SARS-CoV-2 E gene RT-PCR and virus viability as determined by cell culture.

These results demonstrate that infectivity (as defined by growth in cell culture) is significantly reduced when RT-PCR Ct values are > 24. For every 1-unit increase in Ct, the odds ratio for infectivity decreased by 32%. The high specificity of Ct and STT suggests that Ct values > 24, along with duration of symptoms > 8 days, may be used in combination to determine duration of infectivity in patients. Positive cell culture results in our study were most likely between days 1 and 5. This finding is consistent with existing literature [1, 2].

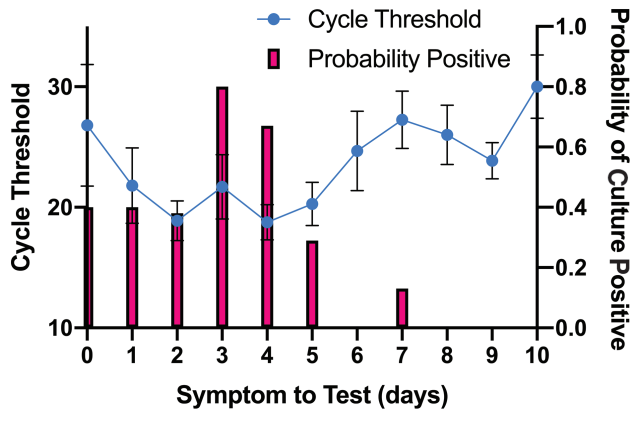

Figure 3. Comparison of symptom onset to test (days) to the probability of successful cultivation on Vero cells (Probability Positive) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) E gene reverse-transcription polymerase chain reaction cycle threshold (Ct) value.

This study is the first to report a large enough data set that demonstrates a link between in vitro viral growth, Ct value, and STT. These results have implications for clinical care, infection prevention and control, and public health. These data can be used to efficiently target case finding efforts by better defining the period of maximal transmission risk. This will be of particular importance in the maintenance phase of the response, where case finding efforts to rapidly interrupt chains of transmission will be essential. Isolation of COVID-19 cases in the community is typically recommended for at least 10 days after symptom onset. Our data supports this approach. Jurisdictions across Canada and the United States are recommending a variety of strategies to discontinue isolation of hospitalized COVID-19 cases [8–13]. Clinical criteria including 14 days from symptom onset or 72 hours symptom free (whichever is longer) are being used in some, while other jurisdictions are using 2 negative NP RT-PCR results 48 hours apart after 14 days of symptoms. Our data support the former approach since RT-PCR positivity persists significantly beyond infectivity; the alternative approach may lead to unnecessary isolation, and use of personal protective equipment and testing resources. The qualitative reporting of results of SARS-CoV-2 RT-PCR as positive or negative is sufficient for diagnosis but may be supplemented by Ct, a semiquantitative value, as well as time of symptom onset to guide infection control, public health, and occupational health decisions.

Our study has important limitations. First, our study utilized a single SARS-CoV-2 gene target (E gene). Though other gene targets may offer greater specificity, the SARS-CoV-2 E gene is more consistently used in both laboratory-developed tests and commercial assays. The testing criteria in Manitoba had sufficient pretest probability to make the likelihood of a false-positive remote. In addition, the first 71 of 90 samples were confirmed using the described protocol with the Centers for Disease Control and Prevention N1 gene target [14]. Second target confirmation was discontinued at that time based on being satisfied with testing criteria and assay sensitivity to accurately identify true COVID-19 cases. Reagent supply also played a role. Second, the recall bias of symptom onset is possible, but this likely would have been equally distributed between those who were culture positive and negative. Third, the infectivity of certain individual cases and the accuracy of our culture assay may have unique variations. Though some individuals in our cross-sectional study would be considered immunocompromised, patients with these conditions could have prolonged shedding of infective SARS-CoV-2 and may not be

fully represented here. Few children have been diagnosed with COVID-19 in our province (median age of positive PCR, 45 [IQR, 30–59] years). With other respiratory viruses, children may have prolonged shedding. Finally, our patient numbers remain small and larger studies are needed to establish Ct criteria that reliably correlate with loss of infectivity and that utilize additional SARS-CoV-2 gene targets.

In conclusion, the SARS-CoV-2/COVID-19 pandemic represents a dynamic situation where decisions and policy must be guided by evidence. Our study showed no positive viral cultures with a Ct > 24 or STT > 8 days. The odds of a positive culture were decreased by 32% for each unit increase in Ct. These data, if confirmed, may help guide isolation, contact tracing, and testing guidelines.

Notes

Acknowledgments. This work was supported by the collaborative efforts in the public health response to the current pandemic by Manitoba Health and Cadham Provincial Laboratory (CPL), and the Public Health Agency of Canada and the National Microbiology Laboratory. A special acknowledgment goes to the medical laboratory technologists in the Virus Detection Section of CPL. We would be blind without you.

Potential conflicts of interest. The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

References

1. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-19. Nature [manuscript published online ahead of print 1 April 2020]. doi:10.1038/s41586-020-2196-x.
2. He X, Lau EHY, Wu P, et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat Med **2020**; 26:672–5.
3. Ip DKM, Lau LLH, Chan KH, et al. The dynamic relationship between clinical symptomatology and viral shedding in naturally acquired seasonal and pandemic influenza virus infections. Clin Infect Dis **2016**; 62:431–7.
4. Falsey AR, Formica MA, Treanor JJ, Walsh EE. Comparison of quantitative reverse transcription-PCR to viral culture for assessment of respiratory syncytial virus shedding. J Clin Microbiol **2003**; 41:4160–5.
5. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill **2020**; 25. doi:10.2807/1560–7917.ES.2020.25.3.2000045.
6. Ramakrishnan MA. Determination of 50% endpoint titer using a simple formula. World J Virol **2016**; 5:85–6. doi:10.5501/wjv.v5.i2.85.
7. Strong JE, Feldmann H. The crux of Ebola diagnostics. J Infect Dis **2017**; 216:1340–2.
8. Government of Canada, Public Health Agency of Canada. Coronavirus disease (COVID-19): for health professionals. Available at: https://www.canada.ca/en/public-health/services/diseases/2019-novel-coronavirus-infection/health-professionals.html. Accessed 24 April 2020.
9. Centers for Disease Control and Prevention. Symptom-based strategy to discontinue isolation for persons with COVID-19. Available at: https://www.cdc.gov/coronavirus/2019-ncov/community/strategy-discontinue-isolation.html. Accessed 5 May 2020.
10. Shared Health Manitoba. Provincial COVID-19 resources for health-care providers and staff. Available at: https://sharedhealthmb.ca/covid19/providers/. Accessed 24 April 2020.
11. Institut national de santé publique Québec (INSPQ). COVID-19 (coronavirus) public health expertise and reference centre. Available at: https://www.inspq.qc.ca/en. Accessed 24 April 2020.
12. Public Health Ontario. COVID-19 health care resources. Available at: https://www.publichealthontario.ca/en/diseases-and-conditions/infectious-diseases/respiratory-diseases/novel-coronavirus/health-care-resources. Accessed 24 April 2020.
13. BC Centre for Disease Control. COVID-19 care. http://www.bccdc.ca/health-professionals/clinical-resources/covid-19-care. Accessed 24 April 2020.
14. Centers for Disease Control and Prevention. Research use only 2019-novel coronavirus (2019-nCoV) real-time RT-PCR primer and probe information (CDC N1 gene). Available at: https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html. Accessed 15 May 2020.

FORM 43A

Rule 43.06(3)

IN THE SUPREME COURT OF VICTORIA AT MELBOURNE
COMMON LAW DIVISON
JUDICIAL REVIEW AND APPEALS LIST

No. S ECI 2021/03031

B E T W E E N

JONATHAN EDWARD KINGSFORD ANDREWS — Plaintiff

-and-

PROFESSOR BRETT SUTTON — First Defendant
(in his capacity as Chief Health Officer as designated under the *COVID Omnibus (Emergency Measures) Act 2020*)

ASSOCIATE PROFESSOR MICHELLE GILES — Second Defendant
(in her capacity as Deputy Health Commander as authorised to exercise emergency powers by Chief Health Officer under section 199(2)9(a) of the *Public Health and Wellbeing Act 2008*)

CERTIFICATE IDENTIFYING EXHIBIT

Date of Document:	24 August 2021	Solicitors Code:	141108
Filed on behalf of:	Jonathan E. K. Andrews	DX:	
Prepared by:	Jonathan E. K. Andrews	Telephone:	(03) 9070 1286
	Bosanquet Solicitors,	Ref:	210801
	TOK Corporate Centre, Level 1	Email:	info@bosanquets.com
	459 Toorak Rd, TOORAK VIC 3142		

This is the exhibit marked "**JA-03**" now produced and shown to JONATHAN EDWARD KINGSFORD ANDREWS at the time of swearing/affirming that person's affidavit on 24 AUGUST 2021:

……

JONATHAN EDWARD KINGSFORD ANDREWS

The Affidavit & Annexures were sworn to and signed by the Deponent by audio visual link and I, as affidavit taker, have used a scanned or electronic copy of the affidavit & annexures and not the original, in completing the jurat requirements under subsection s 27 ss (1) of the Oaths and Affirmations Act 2018 (VIC).

……

Mr Tony Nikolic, Director

Ashley, Francina, Leonard & Associates

Level 25, Tower 3, 300 Barangaroo Avenue,

Sydney, NSW, 2000

(An Australian Legal Practitioner as per Legal Profession Uniform Law [VIC])

Exhibit “JA-03”

External peer review of the RTPCR test to detect SARS-CoV-2.

External peer review of the RTPCR test to detect SARS-CoV-2 reveals 10 major scientific flaws at the molecular and methodological level: consequences for false positive results

Preprint · November 2020

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External peer review of the RTPCR test to detect SARS-CoV-2 reveals 10 major scientific flaws at the molecular and methodological level: consequences for false positive results.

Pieter Borger [1*], Rajesh K. Malhotra [2], Michael Yeadon [3], Clare Craig [4], Kevin McKernan [5]
Klaus Steger [6], Paul McSheehy [7], Lidiya Angelova [8], Fabio Franchi [9], Thomas Binder [10]
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Dorothea Gilbert [15], Rainer J. Klement [16], Ruth Schruefer [17], Berber W. Pieksma [18], Jan Bonte [19],
Bruno H. Dalle Carbonara[20], Kevin P. Corbett [21], Ulrike Kämmerer [22].

* Corresponding author

ABSTRACT

In the publication entitled “Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR” (Eurosurveillance 25(8) 2020) the authors present a diagnostic workflow and RT-qPCR protocol for detection and diagnostics of 2019-nCoV (now known as SARS-CoV-2), which they claim to be validated, as well as being a *robust diagnostic methodology for use in public-health laboratory settings*.

In light of all the consequences resulting from this very publication for societies worldwide, a group of independent researchers performed a point-by-point review of the aforesaid publication in which 1) all components of the presented test design were cross checked, 2) the RT-qPCR protocol-recommendations were assesses w.r.t. good laboratory practice, and 3) parameters examined against relevant scientific literature covering the field.

The published RT-qPCR protocol for detection and diagnostics of 2019-nCoV and the manuscript suffer from numerous technical and scientific errors, including insufficient primer design, a problematic and insufficient RT-qPCR protocol, and the absence of an accurate test validation. Neither the presented test nor the manuscript itself fulfils the requirements for an acceptable scientific publication. Further, serious conflicts of interest of the authors are not mentioned. Finally, the very short timescale between submission and acceptance of the publication (24 hours) signifies that a systematic peer review process was either not performed here, or of problematic poor quality.

We provide compelling evidence of several scientific inadequacies, errors and flaws. Considering the scientific and methodological blemishes presented here, we are confident that the editorial board of Eurosurveillance has no other choice but to retract the publication.

CONCISE REVIEW REPORT

This paper will show numerous serious flaws in the Corman-Drosten paper, the significance of which has led to worldwide misdiagnosis of infections attributed to SARS-CoV-2 and associated with the disease COVID-19. We are confronted with stringent lockdowns which have destroyed many people's lives and livelihoods, limited access to education and these imposed restrictions by governments around the world are a direct attack on people's basic rights and their personal freedoms, resulting in collateral damage for entire economies on a global scale.

There are ten fatal problems with the Corman-Drosten paper which we will outline and explain in greater detail in the following sections.

The first and major issue is that the *novel* Coronavirus SARS-CoV-2 (in the publication named 2019-nCoV and in February 2020 named SARS-CoV-2 by an international consortium of virus experts) is based on *in silico* (theoretical) sequences, supplied by a laboratory in China [1], because at the time neither control material of infectious ("live") or inactivated SARS-CoV-2 nor isolated genomic RNA of the virus was available to the authors. To date no validation has been performed by the authorship based on isolated SARS-CoV-2 viruses or full length RNA thereof.

According to Corman et al.: *"We aimed to develop and deploy robust diagnostic methodology for use in public health laboratory settings without having virus material available."* [1]

The focus here should be placed upon the two stated aims: a) *development* and b) *deployment* of a *diagnostic test for use in public health laboratory settings*. These aims are not achievable without having any actual virus material available (e.g. for determining the infectious viral load). In any case, only a protocol with maximal accuracy can be the mandatory and primary goal in any scenario-outcome of this magnitude. Critical viral load determination is mandatory information, and it is in Christian Drosten's group responsibility to perform these experiments and provide the crucial data.

Nevertheless these *in silico* sequences were used to develop a RT-PCR test methodology to identify the aforesaid virus. This model was based on the assumption that the *novel* virus is very similar to SARS-CoV from 2003 (Hereafter named SARS-CoV-1) as both are beta-coronaviruses.

The PCR test was therefore designed using the genomic sequence of SARS-CoV-1 as a control material for the Sarbeco component; we know this from our personal email-communication with [2] one of the co-authors of the Corman-Drosten paper. This method to model SARS-CoV-2 was described in the Corman-Drosten paper as follows:
"the establishment and validation of a diagnostic workflow for 2019-nCoV screening and specific confirmation, designed in absence of available virus isolates or original patient specimens. Design and validation were enabled by the close genetic relatedness to the 2003 SARS-CoV, and aided by the use of synthetic nucleic acid technology."

In short, a design relying merely on close genetic relatives does not fulfill the aim for a "robust diagnostic test" as cross reactivity and therefore false-positive results will inevitably occur.
Validation was only done in regards to *in silico* (theoretical) sequences and within the laboratory-setting, and not as required for in-vitro diagnostics with isolated genomic viral RNA. This very fact hasn't changed even after 10 months of introduction of the test into routine diagnostics.
There are numerous other severe scientific errors regarding the biomolecular design of the primers, the PCR method, as well as the molecular validation of the PCR products and methods described in the Corman-Drosten paper which are examined in detail in the following chapters. The paper itself already signifies that a large number of false positive results are generated by this test, even under controlled laboratory conditions, making it completely unsuitable as a reliable virus screening method for entire populations in an ongoing pandemic. Given the far-reaching implications, including quarantine measures, lockdowns, curfews and impacts on education etc., this paper must be immediately retracted.

DESIGN AND ERRORS in RT-PCR

The Reverse Transcription-Polymerase Chain Reaction (RT-PCR) is an important biomolecular technology to rapidly detect rare RNA fragments, which are known in advance. In the first step, RNA molecules present in the sample are reverse transcribed to yield cDNA. The cDNA is then amplified in the polymerase chain reaction using a specific primer pair and a thermostable DNA polymerase enzyme. The technology is highly sensitive and its detection limit is theoretically 1 molecule of cDNA. The specificity of the PCR is highly influenced by biomolecular design errors.

What is important when designing an RT-PCR Test and the quantitative RT-qPCR test described in the Corman-Drosten publication?

1. The primers and probes:

   a) the concentration of primers and probes must be of optimal range (100-200 nM)
   b) must be specific to the target-gene you want to amplify
   c) must have an optimal percentage of GC content relative to the total nitrogenous bases (minimum 40%, maximum 60%)
   d) for virus diagnostics at least 3 primer pairs must detect 3 viral genes (preferably as far apart as possible in the viral genome)

2. The temperature at which all reactions take place:

   a) DNA melting temperature (>92°)
   b) DNA amplification temperature (TaqPol specific)
   c) Tm; the annealing temperature (the temperature at which the primers and probes reach the target binding/detachment, not to exceed 2˚C per primer pair).
   Tm heavily depends on GC content of the primers

3. The number of amplification cycles (less than 35; preferably 25-30 cycles); In case of virus detection, >35 cycles only detects signals which do not correlate with infectious virus as determined by isolation in cell culture [reviewed in 2]; if someone is tested by PCR as positive when a threshold of 35 cycles or higher is used (as is the case in most laboratories in Europe & the US), the probability that said person is actually infected is less than 3%, the probability that said result is a false positive is 97%

[reviewed in 3]

4. Molecular biological validations; amplified PCR products must be validated either by running the products in a gel with a DNA ruler, or by direct DNA sequencing

5. Positive and negative controls should be specified to confirm/refute specific virus detection

6. There should be a Standard Operational Procedure (SOP) available, which unequivocally specifies the above parameters, so that all laboratories are able to set up the exact same test conditions. To have a validated universal SOP is essential, because it enables the comparison of data within and between countries.

MINOR CONCERNS WITH THE CORMAN-DROSTEN PAPER

1. In Table 1 of the Corman-Drosten paper, different abbreviations are stated - “nM” is specified, “nm” isn’t. Further in regards to correct nomenclature, nm means “nanometer” therefore nm should read nM here.

2. It is the general consensus to write genetic sequences always in the 5’-3’ direction, including the reverse primers. It is highly unusual to do alignment with reverse complementary writing of the primer sequence as the authors did in figure 2 of the Corman-Drosten paper. Here, in addition, a wobble base is marked as “y” without description of the bases the Y stands for.

3. Two misleading pitfalls in the Corman-Drosten paper are that their Table 1 does not include Tm-values (annealing-temperature values), neither does it show GC-values (number of G and C in the sequences as %-value of total bases).

MAJOR CONCERNS WITH THE CORMAN-DROSTEN PAPER

A) BACKGROUND

The authors introduce the background for their scientific work as: "*The ongoing outbreak of the recently emerged novel coronavirus (2019-nCoV) poses a challenge for public health laboratories as virus isolates are unavailable while there is growing evidence that the outbreak is more widespread than initially thought, and international spread through travelers does already occur".*

According to BBC News [4] and Google Statistics [5] there were 6 deaths world-wide on January 21st 2020 - the day when the manuscript was submitted. Why did the authors assume a challenge for public health laboratories while there was no substantial evidence at that time to indicate that the outbreak was more widespread than initially thought?

As an aim the authors declared to develop and deploy robust diagnostic methodology for use in public health laboratory settings without having virus material available. Further, they acknowledge that "The present study demonstrates the enormous response capacity achieved through coordination of academic and public laboratories in national and European research networks."

B) Methods and Results

1. Primer & Probe Design

1a) Erroneous primer concentrations

Reliable and accurate PCR-test protocols are normally designed using between 100 nM and 200 nM per primer [7]. In the Corman-Drosten paper, we observe unusually high and varying primer concentrations for several primers (table 1). For the RdRp_SARSr-F and RdRp_SARSr-R primer pairs, 600 nM and 800 nM are described, respectively. Similarly, for the N_Sarbeco_F and N_Sarbeco_R primer set, they advise 600 nM and 800 nM, respectively [1]. It should be clear that these concentrations are far too high to be optimal for specific amplifications of target genes. *There exists no specified reason to use these extremely high*

concentrations of primers in this protocol. Rather, these concentrations lead to increased unspecific binding and PCR product amplification.

Table1: Primers and probes (adapted from Corman-Drosten paper; erroneous primer concentrations are highlighted)

Assay/use	Oligonucleotide	Sequence[a]	Concentration[b]
RdRP gene	RdRp_SARSr-F	GTGARATGGTCATGTGTGGCGG	Use 600 nM per reaction
	RdRp_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ	Specific for 2019-nCoV, will not detect SARS-CoV. Use 100 nM per reaction and mix with P1
	RdRP_SARSr-P1	FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ	Pan Sarbeco-Probe will detect 2019-nCoV, SARS-CoV and bat-SARS-related CoVs. Use 100 nM per reaction and mix with P2
	RdRp_SARSr-R	CARATGTTAAASACACTATTAGCATA	Use 800 nM per reaction
E gene	E_Sarbeco_F	ACAGGTACGTTAATAGTTAATAGCGT	Use 400 nm per reaction
	E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ	Use 200 nm per reaction
	E_Sarbeco_R	ATATTGCAGCAGTACGCACACA	Use 400 nm per reaction
N gene	N_Sarbeco_F	CACATTGGCACCCGCAATC	Use 600 nm per reaction
	N_Sarbeco_P	FAM-ACTTCCTCAAGGAACAACATTGCCA-BBQ	Use 200 nm per reaction
	N_Sarbeco_R	GAGGAACGAGAAGAGGCTTG	Use 800 nm per reaction

[a] W is A/T; R is G/A; M is A/C; S is G/C. FAM: 6-carboxyfluorescein; BBQ: blackberry quencher.

[b] Optimised concentrations are given in nanomol per litre (nM) based on the final reaction mix, e.g. 1.5 µL of a 10 µM primer stock solution per 25 µL total reaction volume yields a final concentration of 600 nM as indicated in the table.

1b) Unspecified ("Wobbly") primer and probe sequences

To obtain reproducible and comparable results, it is essential to distinctively define the primer pairs. In the Corman-Drosten paper we observed six unspecified positions, indicated by the letters R, W, M and S (Table 2). The letter W means that at this position there can be either an A or a T; R signifies there can be either a G or an A; M indicates that the position may either be an A or a C; the letter S indicates there can be either a G or a C on this position.

This high number of variants not only is unusual, but it also is highly confusing for laboratories. These six unspecified positions could easily result in the design of several different alternative primer sequences which do not relate to SARS-CoV-2 (2 distinct RdRp_SARSr_F primers + 8 distinct RdRp_SARS_P1 probes + 4 distinct RdRp_SARSr_R). *The design variations will inevitably lead to results that are not even SARS-CoV-2 related. Therefore, the confusing unspecific description in the Corman-Drosten paper is not suitable as a Standard Operational Protocol. These unspecified positions should have been designed unequivocally.*

These wobbly sequences have already created a source of concern in the field and resulted in a Letter to the Editor authored by Pillonel *et al.* [8] regarding blatant errors in the described sequences. These errors are self-evident in the Corman *et al.* supplement as well.

Table 2: Primers and probes (adapted from Corman-Drosten paper; unspecified ("Wobbly") nucleotides in the primers are highlighted)

Assay/use	Oligonucleotide	Sequence[a]	Concentration[b]
RdRP gene	RdRp_SARSr-F	GTGARATGGTCATGTGTGGCGG	Use 600 nM per reaction
	RdRp_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ	Specific for 2019-nCoV, will not detect SARS-CoV. Use 100 nM per reaction and mix with P1
	RdRP_SARSr-P1	FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ	Pan Sarbeco-Probe will detect 2019-nCoV, SARS-CoV and bat-SARS-related CoVs. Use 100 nM per reaction and mix with P2
	RdRp_SARSr-R	CARATGTTAAASACACTATTAGCATA	Use 800 nM per reaction
E gene	E_Sarbeco_F	ACAGGTACGTTAATAGTTAATAGCGT	Use 400 nm per reaction
	E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ	Use 200 nm per reaction
	E_Sarbeco_R	ATATTGCAGCAGTACGCACACA	Use 400 nm per reaction
N gene	N_Sarbeco_F	CACATTGGCACCCGCAATC	Use 600 nm per reaction
	N_Sarbeco_P	FAM-ACTTCCTCAAGGAACAACATTGCCA-BBQ	Use 200 nm per reaction
	N_Sarbeco_R	GAGGAACGAGAAGAGGCTTG	Use 800 nm per reaction

W is A/T; R is G/A; M is A/C; S is G/C; FAM: 6-carboxyfluorescein; BBQ: blackberry quencher.

[b] Optimised concentrations are given in nanomol per litre (nM) based on the final reaction mix, e.g. 1.5 µL of a 10 µM primer stock solution per 25 µL total reaction volume yields a final concentration of 600 nM as indicated in the table.

The WHO-protocol (Figure 1), which directly derives from the Corman-Drosten paper, concludes that in order to confirm the presence of SARS-CoV-2, two control genes (the E- and the RdRp-genes) must be identified in the assay. It should be noted, that the RdPd-gene has one uncertain position ("wobbly") in the forward-primer (R=G/A), two uncertain positions in the reverse-primer (R=G/A; S=G/C) and it has three uncertain positions in the RdRp-probe (W=A/T; R=G/A; M=A/C). So, two different forward primers, four different reverse primers, and eight distinct probes can be synthesized for the RdPd-gene. Together, there are 64 possible combinations of primers and probes!

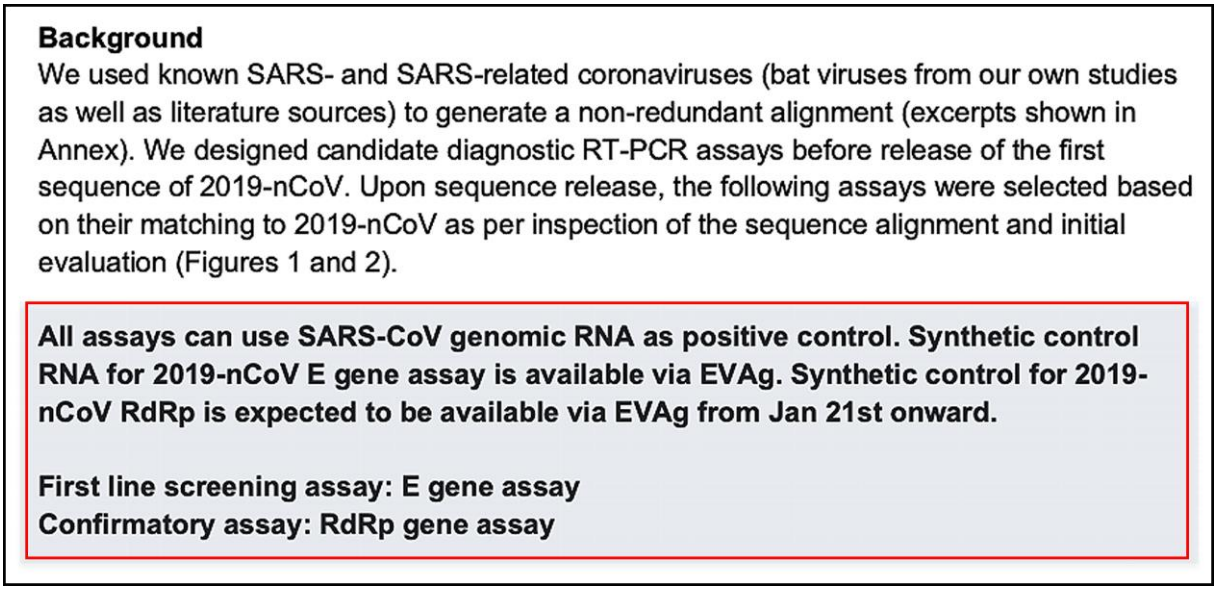
The Corman-Drosten paper further identifies a third gene which, according to the WHO protocol, was not further validated and deemed unnecessary: *"Of note, the N gene assay also performed well but was not subjected to intensive further validation because it was slightly less sensitive."*

This was an unfortunate omission as it would be best to use all three gene PCRs as

confirmatory assays, and this would have resulted in an almost sufficient virus RNA detection diagnostic tool protocol. Three confirmatory assay-steps would at least minimize-out errors & uncertainties at every fold-step in regards to "Wobbly"-spots. (Nonetheless, the protocol would still fall short of any "good laboratory practice", when factoring in all the other design-errors).

As it stands, the N gene assay is regrettably neither proposed in the WHO-recommendation (Figure 1) as a mandatory and crucial third confirmatory step, nor is it emphasized in the Corman-Drosten paper as important optional reassurance "for a routine workflow" (Table 2).

Consequently, in nearly all test procedures worldwide, merely 2 primer-matches were used instead of all three. This oversight renders the entire test-protocol useless with regards to delivering accurate test-results of real significance in an ongoing pandemic.



Background

We used known SARS- and SARS-related coronaviruses (bat viruses from our own studies as well as literature sources) to generate a non-redundant alignment (excerpts shown in Annex). We designed candidate diagnostic RT-PCR assays before release of the first sequence of 2019-nCoV. Upon sequence release, the following assays were selected based on their matching to 2019-nCoV as per inspection of the sequence alignment and initial evaluation (Figures 1 and 2).

All assays can use SARS-CoV genomic RNA as positive control. Synthetic control RNA for 2019-nCoV E gene assay is available via EVAg. Synthetic control for 2019-nCoV RdRp is expected to be available via EVAg from Jan 21st onward.

First line screening assay: E gene assay
Confirmatory assay: RdRp gene assay

Figure 1: The N-Gene confirmatory-assay is neither emphasized as necessary third step in the official WHO Drosten-Corman protocol-recommendation [8] nor is it required as a crucial step for higher test-accuracy in the Eurosurveillance publication.

1c) Erroneous GC-content (discussed in 2c, together with annealing temperature (Tm))

1d) Detection of viral genes

RT-PCR is not recommended for primary diagnostics of infection. This is why the RT-PCR Test

used in clinical routine for detection of COVID-19 is not indicated for COVID-19 diagnosis on a regulatory basis.

"Clinicians need to recognize the enhanced accuracy and speed of the molecular diagnostic techniques for the diagnosis of infections, but also to understand their limitations. Laboratory results should always be interpreted in the context of the clinical presentation of the patient, and appropriate site, quality, and timing of specimen collection are required for reliable test results". [9]

However, it may be used to help the physician's differential diagnosis when he or she has to discriminate between different infections of the lung (Flu, Covid-19 and SARS have very similar symptoms). For a confirmative diagnosis of a specific virus, at least 3 specific primer pairs must be applied to detect 3 virus-specific genes. Preferably, these target genes should be located with the greatest distance possible in the viral genome (opposite ends included). Although the Corman-Drosten paper describes 3 primers, these primers only cover roughly half of the virus' genome. This is another factor that decreases specificity for detection of intact COVID-19 virus RNA and increases the quote of false positive test results.

Therefore, even if we obtain three positive signals (i.e. the three primer pairs give 3 different amplification products) in a sample, this does not prove the presence of a virus. A better primer design would have terminal primers on both ends of the viral genome. This is because the whole viral genome would be covered and three positive signals can better discriminate between a complete (and thus potentially infectious) virus and fragmented viral genomes (without infectious potency). In order to infer anything of significance about the infectivity of the virus, the Orf1 gene, which encodes the essential replicase enzyme of SARS-CoV-1 and SARS-CoV-2 viruses, should have been included as a target (Figure 2). The positioning of the targets in the region of the viral genome that is most heavily and variably transcribed is another weakness of the protocol.

Kim *et al.* demonstrate a highly variable 3' expression of subgenomic RNA in Sars-CoV-2 [23]. These RNAs are actively monitored as signatures for asymptomatic and non-infectious patients [10]. It is highly questionable to screen a population of asymptomatic people with qPCR primers that have 6 base pairs primer-dimer on the 3 prime end of a primer (Figure 3). Apparently the WHO recommends these primers. We tested all the wobble derivatives from

the Corman-Drosten paper with Thermofisher's primer dimer web tool [11]. The RdRp forward primer has 6bp 3prime homology with Sarbeco E Reverse. At high primer concentrations this is enough to create inaccuracies.

Of note: There is a perfect match of one of the N primers to a clinical pathogen (*Pantoea*), found in immuno-compromised patients. The reverse primer hits *Pantoea* as well but not in the same region (Figure 3).

<u>These are severe design errors, since the test cannot discriminate between the whole virus and viral fragments. The test cannot be used as a diagnostic for SARS-CoV-2 viruses.</u>

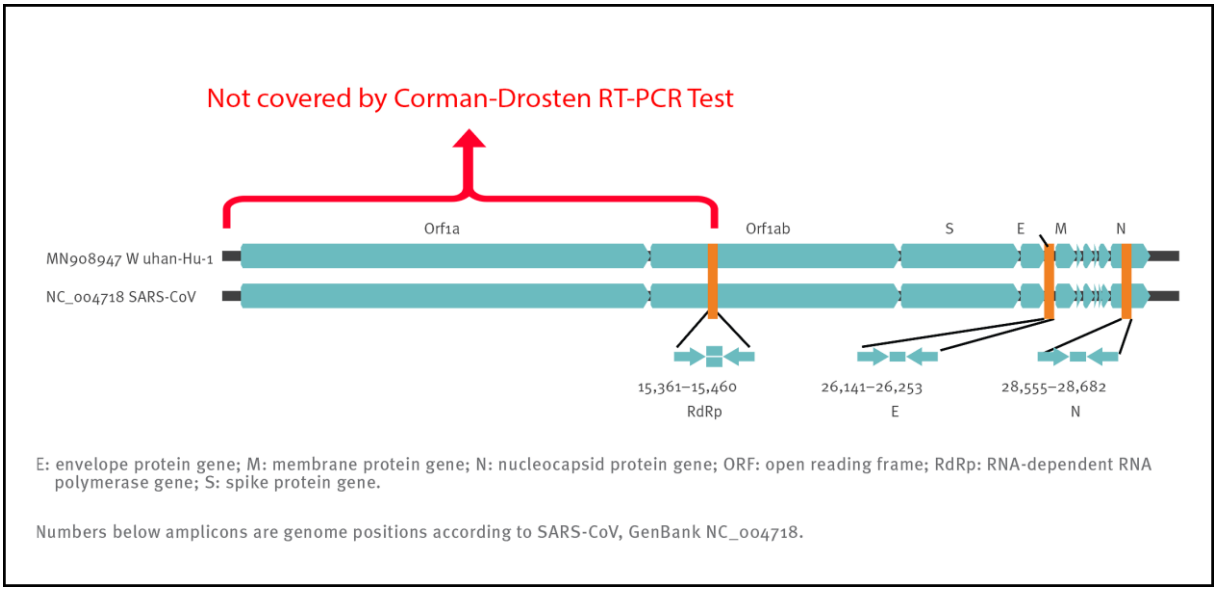


Figure 2: Relative positions of amplicon targets on the SARS-CoV-1 coronavirus and the 2019 novel coronavirus genome. ORF: open reading frame; RdRp: RNA-dependent RNA polymerase. Numbers below amplicon are genome positions according to SARS-CoV-1, NC_004718 [1];

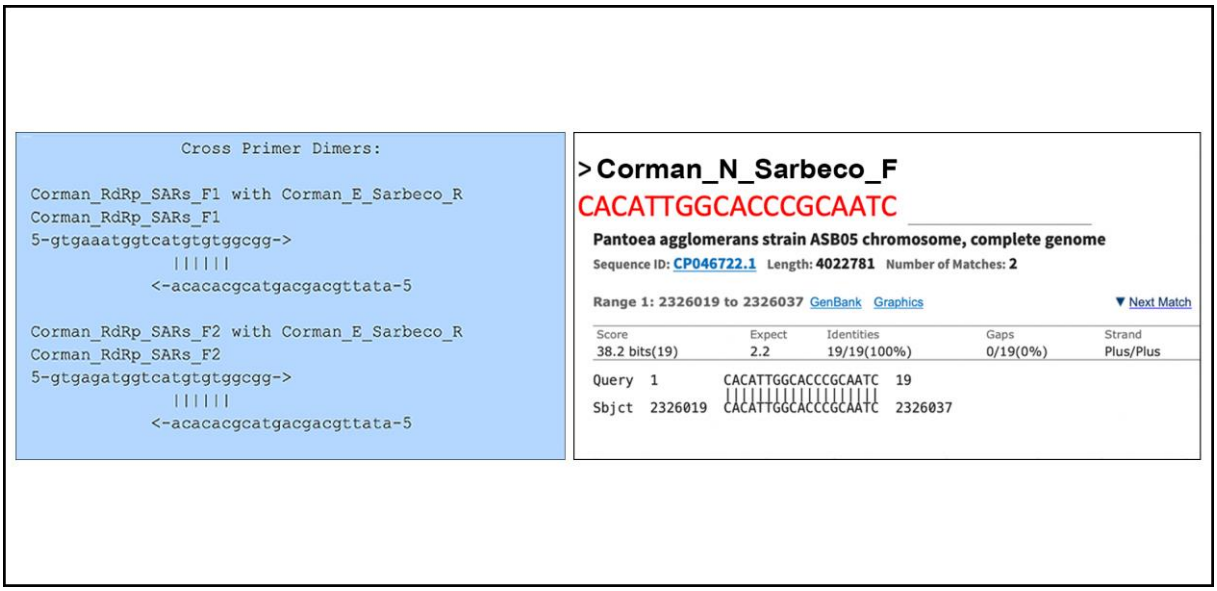


Figure 3: A test with Thermofischer's primer dimer web tool reveals that the RdRp forward primer has a 6bp 3`prime homology with Sarbeco E Reverse (left box). Another test reveals that there is a perfect match for one of the N-primers to a clinical pathogen (*Pantoea*) found in immuno-compromised patients (right box).

2. Reaction temperatures

2a) DNA melting temperature (>92°).

Adequately addressed in the Corman-Drosten paper.

2b) DNA amplification temperature.

Adequately addressed in the Corman-Drosten paper.

2c) Erroneous GC-contents and Tm

The annealing-temperature determines at which temperature the primer attaches/detaches from the target sequence. For an efficient and specific amplification, GC content of primers should meet a minimum of 40% and a maximum of 60% amplification. As indicated in table 3, three of the primers described in the Corman-Drosten paper are not within the normal range for GC-content. Two primers (RdRp_SARSr_F and RdRp_SARSr_R) have unusual and very low GC-values of 28%-31% for all possible variants of wobble bases, whereas primer E_Sarbeco_F has a GC-value of 34.6% (Table 3 and second panel of Table 3).

It should be noted that the GC-content largely determines the binding to its specific target due to its three hydrogen bonds in base pairing. Thus, the lower the GC-content of the primer, the lower its binding-capability to its specific target gene sequence (i.e. the gene to

be detected). This means for a target-sequence to be recognized we have to choose a temperature which is as close as possible to the actual annealing-temperature (best practise-value) for the primer not to detach again, while at the same time specifically selecting the target sequence.

If the Tm-value is very low, as observed for all wobbly-variants of the RdRp reverse primers, the primers can bind non-specifically to several targets, decreasing specificity and increasing potential false positive results.

The annealing temperature (Tm) is a crucial factor for the determination of the specificity /accuracy of the qPCR procedure and essential for evaluating the accuracy of qPCR-protocols. Best-practice recommendation: Both primers (forward and reverse) should have an almost similar value, preferably the identical value.

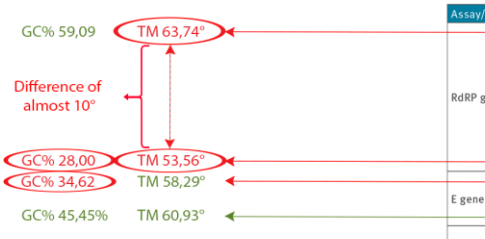
We used the freely available primer design software Primer-BLAST [12, 25] to evaluable the best-practise values for all primers used in the Corman-Drosten paper (Table 3). We attempted to find a Tm-value of 60° C, while similarly seeking the highest possible GC%-value for all primers. A maximal Tm difference of 2° C within primer pairs was considered acceptable. Testing the primer pairs specified in the Corman-Drosten paper, we observed a difference of 10° C with respect to the annealing temperature Tm for primer pair1 (RdRp_SARSr_F and RdRp_SARSr_R). *This is a very serious error and makes the protocol useless as a specific diagnostic tool.*

Additional testing demonstrated that only the primer pair designed to amplify the N-gene (N_Sarbeco_F and N_Sarbeco_R) reached the adequate standard to operate in a diagnostic test, since it has a sufficient GC-content and the Tm difference between the primers (N_Sarbeco_F and N_Sarbeco_R) is 1.85° C (below the crucial maximum of 2° C difference). Importantly, this is the gene which was neither tested in the virus samples (Table 2) nor emphasized as a confirmatory test. In addition to highly variable melting temperatures and degenerate sequences in these primers, there is another factor impacting specificity of the procedure: the dNTPs (0.4uM) are 2x higher than recommended for a highly specific amplification. There is additional magnesium sulphate added to the reaction as well. This procedure combined with a low annealing temperature can create non-specific amplifications. When additional magnesium is required for qPCR, specificity of the assay should be further scrutinized.

The design errors described here are so severe that it is highly unlikely that specific amplification of SARS-CoV-2 genetic material will occur using the protocol of the Corman-Drosten paper.

Table 3: GC-content of the primers and probes (adapted from Corman-Drosten paper; aberrations from optimized GC-contents are highlighted. Second Panel shows a table-listing of all Primer-BLAST best practices values for all primers and probes used in the Corman-Drosten paper by Prof. Dr. Ulrike Kämmerer & her team

Normal ranges for GC%: 40 - 60%; normal ranges for TM: 55-65°; Best-practise for qPCR in our case: 60° for both primers (reverse & forward)



Assay/use	Oligonucleotide	Sequence[a]	Concentration[b]
RdRP gene	RdRp_SARSr-F	GTGARATGGTCATGTGTGGCGG	Use 600 nM per reaction
	RdRp_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ	Specific for 2019-nCoV, will not detect SARS-CoV. Use 100 nM per reaction and mix with P1
	RdRP_SARSr-P1	FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ	Pan Sarbeco-Probe will detect 2019-nCoV, SARS-CoV and bat-SARS-related CoVs. Use 100 nM per reaction and mix with P2
	RdRp_SARSr-R	CARATGTTAAASACACTATTAGCATA	Use 800 nM per reaction
E gene	E_Sarbeco_F	ACAGGTACGTTAATAGTTAATAGCGT	Use 400 nm per reaction
	E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ	Use 200 nm per reaction
	E_Sarbeco_R	ATATTGCAGCAGTACGCACACA	Use 400 nm per reaction
N gene	N_Sarbeco_F	CACATTGGCACCCGCAATC	Use 600 nm per reaction
	N_Sarbeco_P	FAM-ACTTCCTCAAGGAACAACATTGCCA-BBQ	Use 200 nm per reaction
	N_Sarbeco_R	GAGGAACGAGAAGAGGCTTG	Use 800 nm per reaction

[a] W is A/T; R is G/A; M is A/C; S is G/C. FAM: 6-carboxyfluorescein; BBQ: blackberry quencher.
[b] Optimised concentrations are given in nanomol per litre (nM) based on the final reaction mix, e.g. 1.5 µL of a 10 µM primer stock solution per 25 µL total reaction volume yields a final concentration of 600 nM as indicated in the table.

		GC	TM	Search in **MN908947** (first full genome from Wuihan, 12.01.2020)						
Primer pairs	**Sequence (5´-3´)**	**Template strand**	**Length**	**Start**	**Stop**	**Tm**	**GC%**	**Self 5' complementarity**	**Self 3' complementarity**	**Product length (bp)**
E_Sarbeco_F	ACAGGTACGTTAATAGTTAATAGCGT	Plus	26	26269	26294	58.29	34.62	8.00	8.00	**113**
E_Sarbeco_R	ATATTGCAGCAGTACGCACACA	Minus	22	26381	26360	60.93	45.45	7.00	1.00	
N-Sarbeco_F	CACATTGGCACCCGCAATC	Plus	19	28706	28724	60.15	57.89	4.00	0.00	**128**
N-Sarbeco_R	GAGGAACGAGAAGAGGCTTG	Minus	20	28833	28814	58.00	55.00	3.00	1.00	
RdRp_SARSr-F	GTGARATGGTCATGTGTGGCGG		22			63.74	59.09	4.00	to be added in next version	
RdRp_SARSr-R	CARATGTTAAASACACTATTAGCATA		25			53.56	28.00	7.00		
If R= G and S= G	GTGAGATGGTCATGTGTGGCGG		22			63.74	59.09	4.00	1.00	
	CAGATGTTAAAGACACTATTAGCATA		26			55.22	30.77	7.00	5.00	not found in the Sequence
If R= G and S= C	GTGAGATGGTCATGTGTGGCGG		22			63.74	59.09	4.00	1.00	
	CAGATGTTAAACACACTATTAGCATA		26			55.68	30.77	7.00	2.00	
If R= A and S= G	GTGAAATGGTCATGTGTGGCGG		22			62.58	54.55	4.00	1.00	
	CAAATGTTAAAGACACTATTAGCATA		26			54.23	26.92	7.00	5.00	
If R= A and S= C	GTGAAATGGTCATGTGTGGCGG		22			62.58	54.55	4.00	1.00	
	CAAATGTTAAACACACTATTAGCATA		26			54.69	26.92	7.00	2.00	
Probes:										
RdRp-SARSr-P2	CAGGTGGAACCTCATCAGGAGATGC		25			64.89	56.00	6.00	5.00	
RdRp-SARSr-P1	CCAGGTGGWACRTCATCMGGTGATGC									
E-Sarbeco-P1	ACACTAGCCATCCTTACTGCGCTTCG		26			66.78	53.85	4.00	2.00	
N-Sarbeco-P	ACTTCCTCAAGGAACAACATTGCCA		25			63.15	44.00	8.00	3.00	

3. The number of amplification cycles

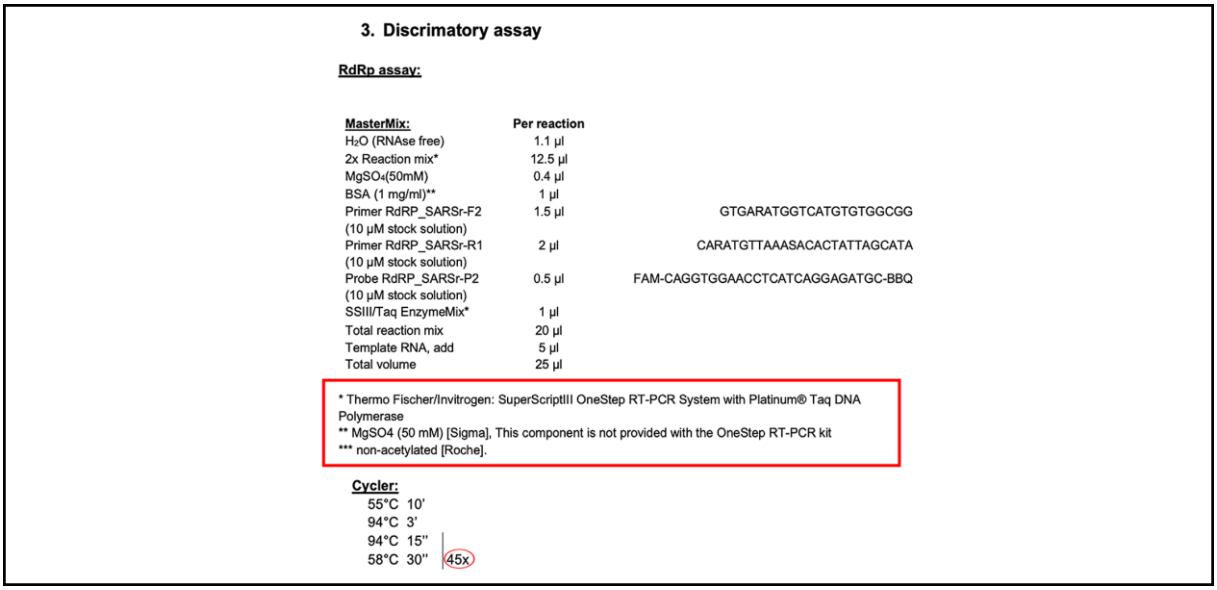
It should be noted that there is no mention anywhere in the Corman-Drosten paper of a test being positive or negative, or indeed what defines a positive or negative result. These types of virological diagnostic tests must be based on a SOP, including a validated and fixed number of PCR cycles (Ct value) after which a sample is deemed positive or negative. The maximum reasonably reliable Ct value is 30 cycles. Above a Ct of 35 cycles, rapidly increasing numbers of false positives must be expected .

PCR data evaluated as positive after a Ct value of 35 cycles are completely unreliable.

Citing Jaafar *et al.* 2020 [3]: *"At Ct = 35, the value we used to report a positive result for PCR, <3% of cultures are positive."* In other words, there was no successful virus isolation of SARS-CoV-2 at those high Ct values.

Further, scientific studies show that only non-infectious (dead) viruses are detected with Ct values of 35 [22].

Between 30 and 35 there is a grey area, where a positive test cannot be established with certainty. This area should be excluded. Of course, one could perform 45 PCR cycles, as recommended in the Corman-Drosten WHO-protocol (Figure 4), but then you also have to define a reasonable Ct-value (which should not exceed 30). But an analytical result with a Ct value of 45 is scientifically and diagnostically absolutely meaningless (a reasonable Ct-value should not exceed 30). All this should be communicated very clearly. *It is a significant mistake that the Corman-Drosten paper does not mention the maximum Ct value at which a sample can be unambiguously considered as a positive or a negative test-result. This important cycle threshold limit is also not specified in any follow-up submissions to date.*



3. Discrimatory assay

RdRp assay:

MasterMix:	Per reaction	
H_2O (RNAse free)	1.1 µl	
2x Reaction mix*	12.5 µl	
$MgSO_4$(50mM)	0.4 µl	
BSA (1 mg/ml)**	1 µl	
Primer RdRP_SARSr-F2 (10 µM stock solution)	1.5 µl	GTGARATGGTCATGTGTGGCGG
Primer RdRP_SARSr-R1 (10 µM stock solution)	2 µl	CARATGTTAAASACACTATTAGCATA
Probe RdRP_SARSr-P2 (10 µM stock solution)	0.5 µl	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ
SSIII/Taq EnzymeMix*	1 µl	
Total reaction mix	20 µl	
Template RNA, add	5 µl	
Total volume	25 µl	

* Thermo Fischer/Invitrogen: SuperScriptIII OneStep RT-PCR System with Platinum® Taq DNA Polymerase
** MgSO4 (50 mM) [Sigma], This component is not provided with the OneStep RT-PCR kit
*** non-acetylated [Roche].

Cycler:
55°C 10'
94°C 3'
94°C 15"
58°C 30" 45x

Figure 4: RT-PCR Kit recommendation in the official Corman-Drosten WHO-protocol [8]. Only a "Cycler"-value (cycles) is to be found without corresponding and scientifically reasonable Ct (Cutoff-value). This or any other cycles-value is nowhere to be found in the actual Corman-Drosten paper.

4. Biomolecular validations

To determine whether the amplified products are indeed SARS-CoV-2 genes, biomolecular validation of amplified PCR products is essential. For a diagnostic test, this validation is an absolute must.

Validation of PCR products should be performed by either running the PCR product in a 1% agarose-EtBr gel together with a size indicator (DNA ruler or DNA ladder) so that the size of the product can be estimated. The size must correspond to the calculated size of the amplification product. But it is even better to sequence the amplification product. The latter will give 100% certainty about the identity of the amplification product. Without molecular validation one can not be sure about the identity of the amplified PCR products. Considering the severe design errors described earlier, the amplified PCR products can be anything.

Also not mentioned in the Corman-Drosten paper is the case of small fragments of qPCR (around 100bp): It could be either 1,5% agarose gel or even an acrylamide gel.
The fact that these PCR products have not been validated at molecular level is another striking error of the protocol, making any test based upon it useless as a specific diagnostic tool to identify the SARS-CoV-2 virus.

5. Positive and negative controls to confirm/refute specific virus detection.

The unconfirmed assumption described in the Corman-Drosten paper is that SARS-CoV-2 is the only virus from the SARS-like beta-coronavirus group that currently causes infections in humans. The sequences on which their PCR method is based are *in silico* sequences, supplied by a laboratory in China [23], because at the time of development of the PCR test no control material of infectious ("live") or inactivated SARS-CoV-2 was available to the authors. The PCR test was therefore designed using the sequence of the known SARS-CoV-1 as a control material for the Sarbeco component (Dr. Meijer, co-author Corman-Drosten paper in an email exchange with Dr. Peter Borger) [2].

All individuals testing positive with the RT-PCR test, as described in the Corman-Drosten paper, are assumed to be positive for SARS-CoV-2 infections. There are three severe flaws in their assumption. First, a positive test for the RNA molecules described in the Corman-Drosten paper cannot be equated to "infection with a virus". A positive RT-PCR test merely indicates the presence of viral RNA molecules. As demonstrated under point 1d (above), the Corman-Drosten test was not designed to detect the full-length virus, but only a fragment of the virus. We already concluded that this classifies the test as unsuitable as a diagnostic test for SARS-virus infections.

Secondly and of major relevance, the functionality of the published RT-PCR Test was not demonstrated with the use of a positive control (isolated SARS-CoV-2 RNA) which is an essential scientific gold standard.

Third, the Corman-Drosten paper states:

"To show that the assays can detect other bat-associated SARS-related viruses, we used the E gene assay to test six bat-derived faecal samples available from Drexler et al. [...] und Muth et al. […]. These virus-positive samples stemmed from European rhinolophid bats. Detection of these phylogenetic outliers within the SARS-related CoV clade suggests that all Asian viruses are likely to be detected. This would, theoretically, ensure broad sensitivity even in case of multiple independent acquisitions of variant viruses from an animal reservoir."

This statement demonstrates that the E gene used in RT-PCR test, as described in the Corman-Drosten paper, is not specific to SARS-CoV-2. The E gene primers also detect a broad spectrum of other SARS viruses.

The genome of the coronavirus is the largest of all RNA viruses that infect humans and they all have a very similar molecular structure. Still, SARS-CoV-1 and SARS-CoV-2 have two highly specific genetic fingerprints, which set them apart from the other coronaviruses. First, a unique fingerprint-sequence (KTFPPTEPKKDKKKK) is present in the N-protein of SARS-CoV-1 and SARS-CoV-2 [13,14,15]. Second, both SARS-CoV-1 and SARS-CoV-2 do not contain the HE protein, whereas all other coronaviruses possess this gene [13, 14]. So, in order to specifically detect a SARS-CoV-1 and SARS-CoV-2 PCR product the above region in the N gene should have been chosen as the amplification target. A reliable diagnostic test should focus on this specific region in the N gene as a confirmatory test. The PCR for this N gene was not further validated nor recommended as a test gene by the Drosten-Corman paper, because of

being “not so sensitive” with the SARS-CoV original probe [1].

Furthermore, the absence of the HE gene in both SARS-CoV-1 and SARS-CoV-2 makes this gene the ideal negative control to exclude other coronaviruses. The Corman-Drosten paper does not contain this negative control, nor does it contain any other negative controls. The PCR test in the Corman-Drosten paper therefore contains neither a unique positive control nor a negative control to exclude the presence of other coronaviruses. This is another major design flaw which classifies the test as unsuitable for diagnosis.

6. Standard Operational Procedure (SOP) is not available

There should be a Standard Operational Procedure (SOP) available, which unequivocally specifies the above parameters, so that all laboratories are able to set up the identical same test conditions. To have a validated universal SOP is essential, because it facilitates data comparison within and between countries. It is very important to specify all primer parameters unequivocally. We note that this has not been done. Further, the Ct value to indicate when a sample should be considered positive or negative is not specified. It is also not specified when a sample is considered infected with SARS-CoV viruses. As shown above, the test cannot discern between virus and virus fragments, so the Ct value indicating positivity is crucially important. This Ct value should have been specified in the Standard Operational Procedure (SOP) and put on-line so that all laboratories carrying out this test have exactly the same boundary conditions. It points to flawed science that such an SOP does not exist. The laboratories are thus free to conduct the test as they consider appropriate, resulting in an enormous amount of variation. Laboratories all over Europe are left with a multitude of questions; which primers to order? which nucleotides to fill in the undefined places? which Tm value to choose? How many PCR cycles to run? At what Ct value is the sample positive? And when is it negative? And how many genes to test? Should all genes be tested, or just the E and RpRd gene as shown in Table 2 of the Corman-Drosten paper? Should the N gene be tested as well? And what is their negative control? What is their positive control? The protocol as described is unfortunately very vague and erroneous in its design that one can go in dozens of different directions. There does not appear to be any standardization nor an SOP, so it is not clear how this test can be implemented.

7. Consequences of the errors described under 1-5: false positive results.

The RT-PCR test described in the Corman-Drosten paper contains so many molecular biological design errors (see 1-5) that it is not possible to obtain unambiguous results. It is inevitable that this test will generate a tremendous number of so-called “false positives”. The definition of false positives is a negative sample, which initially scores positive, but which is negative after retesting with the same test. False positives are erroneous positive test-results, i.e. negative samples that test positive. And this is indeed what is found in the Corman-Drosten paper. On page 6 of the manuscript PDF the authors demonstrate, that even under well-controlled laboratory conditions, a considerable percentage of false positives is generated with this test:

“In four individual test reactions, weak initial reactivity was seen however they were negative upon retesting with the same assay. These signals were not associated with any particular virus, and for each virus with which initial positive reactivity occurred, there were other samples that contained the same virus at a higher concentration but did not test positive. Given the results from the extensive technical qualification described above, it was concluded that this initial reactivity was not due to chemical instability of real-time PCR probes and most probably to handling issues caused by the rapid introduction of new diagnostic tests and controls during this evaluation study.” [1]

The first sentence of this excerpt is clear evidence that the PCR test described in the Corman-Drosten paper generates false positives. Even under the well-controlled conditions of the state-of-the-art Charité-laboratory, 4 out of 310 primary-tests are false positives per definition. Four negative samples initially tested positive, then were negative upon retesting. This is the classical example of a false positive. In this case the authors do not identify them as false positives, which is intellectually dishonest.

Another telltale observation in the excerpt above is that the authors explain the false positives away as "handling issues caused by the rapid introduction of new diagnostic tests". Imagine the laboratories that have to introduce the test without all the necessary information normally described in an SOP.

8. The Corman-Drosten paper was not peer-reviewed

Before formal publication in a scholarly journal, scientific and medical articles are traditionally certified by "peer review." In this process, the journal's editors take advice from various experts ("referees") who have assessed the paper and may identify weaknesses in its assumptions, methods, and conclusions. Typically a journal will only publish an article once the editors are satisfied that the authors have addressed referees' concerns and that the data presented supports the conclusions drawn in the paper." This process is as well described for Eurosurveillance [16].

The Corman-Drosten paper was submitted to Eurosurveillance on January 21st 2020 and accepted for publication on January 22nd 2020. On January 23rd 2020 the paper was online. On January 13th 2020 version 1-0 of the protocol was published at the official WHO website [17], updated on January 17th 2020 as document version 2-1 [18], even before the Corman-Drosten paper was published on January 23rd at Eurosurveillance.

Normally, peer review is a time-consuming process since at least two experts from the field have to critically read and comment on the submitted paper. In our opinion, this paper was not peer-reviewed. Twenty-four hours are simply not enough to carry out a thorough peer review. Our conclusion is supported by the fact that a tremendous number of very serious design flaws were found by us, which make the PCR test completely unsuitable as a diagnostic tool to identify the SARS-CoV-2 virus. Any molecular biologist familiar with RT-PCR design would have easily observed the grave errors present in the Corman-Drosten paper before the actual review process. We asked Eurosurveillance on October 26th 2020 to send us a copy of the peer review report. To date, we have not received this report and in a letter dated November 18th 2020, the ECDC as host for Eurosurveillance declined to provide access without providing substantial scientific reasons for their decision. On the contrary, they write that "disclosure would undermine the purpose of scientific investigations." [24].

9. Authors as the editors

A final point is one of major concern. It turns out that two authors of the Corman-Drosten paper, Christian Drosten and Chantal Reusken, are also members of the editorial board of this journal [19]. Hence there is a severe conflict of interest which strengthens suspicions

that the paper was not peer-reviewed. It has the appearance that the rapid publication was possible simply because the authors were also part of the editorial board at Eurosurveillance. This practice is categorized as compromising scientific integrity .

SUMMARY CATALOGUE OF ERRORS FOUND IN THE PAPER

The Corman-Drosten paper contains the following specific errors:

1. There exists no specified reason to use these extremely high concentrations of primers in this protocol. The described concentrations lead to increased nonspecific bindings and PCR product amplifications, making the test unsuitable as a specific diagnostic tool to identify the SARS-CoV-2 virus.

2. Six unspecified wobbly positions will introduce an enormous variability in the real world laboratory implementations of this test; the confusing nonspecific description in the Corman-Drosten paper is not suitable as a Standard Operational Protocol making the test unsuitable as a specific diagnostic tool to identify the SARS-CoV-2 virus.

3. The test cannot discriminate between the whole virus and viral fragments. Therefore, the test cannot be used as a diagnostic for intact (infectious) viruses, making the test unsuitable as a specific diagnostic tool to identify the SARS-CoV-2 virus and make inferences about the presence of an infection.

4. A difference of 10° C with respect to the annealing temperature Tm for primer pair1 (RdRp_SARSr_F and RdRp_SARSr_R) also makes the test unsuitable as a specific diagnostic tool to identify the SARS-CoV-2 virus.

5. A severe error is the omission of a Ct value at which a sample is considered positive and negative. This Ct value is also not found in follow-up submissions making the test unsuitable as a specific diagnostic tool to identify the SARS-CoV-2 virus.

6. The PCR products have not been validated at the molecular level. This fact makes the protocol useless as a specific diagnostic tool to identify the SARS-CoV-2 virus.

7. The PCR test contains neither a unique positive control to evaluate its specificity for SARS-CoV-2 nor a negative control to exclude the presence of other coronaviruses, making the test unsuitable as a specific diagnostic tool to identify the SARS-CoV-2 virus.

8. The test design in the Corman-Drosten paper is so vague and flawed that one can go in dozens of different directions; nothing is standardized and there is no SOP. This highly questions the scientific validity of the test and makes it unsuitable as a specific diagnostic tool to identify the SARS-CoV-2 virus.

9. Most likely, the Corman-Drosten paper was not peer-reviewed making the test unsuitable as a specific diagnostic tool to identify the SARS-CoV-2 virus.

10. We find severe conflicts of interest for at least four authors, in addition to the fact that two of the authors of the Corman-Drosten paper (Christian Drosten and Chantal Reusken) are members of the editorial board of Eurosurveillance. A conflict of interest was added on July 29 2020 (Olfert Landt is CEO of TIB-Molbiol; Marco Kaiser is senior researcher at GenExpress and serves as scientific advisor for TIB-Molbiol), that was not declared in the original version (and still is missing in the PubMed version); TIB-Molbiol is the company which was “the first” to produce PCR kits (Light Mix) based on the protocol published in the Corman-Drosten manuscript, and according to their own words, they distributed these PCR-test kits before the publication was even submitted [20]; further, Victor Corman & Christian Drosten failed to mention their second affiliation: the commercial test laboratory “Labor Berlin”. Both are responsible for the virus diagnostics there [21] and the company operates in the realm of real time PCR-testing.

CONCLUSION

In light of our re-examination of the test protocol to identify SARS-CoV-2 described in the Corman-Drosten paper we have identified concerning errors and inherent fallacies which render the SARS-CoV-2 PCR test useless.

The decision as to which test protocols are published and made widely available lies squarely in the hands of Eurosurveillance. A decision to recognise the errors apparent in the Corman-Drosten paper has the benefit to greatly minimise human cost and suffering going forward. Is it not in the best interest of Eurosurveillance to retract this paper? Our conclusion is clear. In the face of all the tremendous PCR-protocol design flaws and errors described here, we conclude: There is not much of a choice left in the framework of scientific integrity and responsibility.

References

[1] Corman Victor M, Landt Olfert, Kaiser Marco, Molenkamp Richard, Meijer Adam, Chu Daniel KW, Bleicker Tobias, Brünink Sebastian, Schneider Julia, Schmidt Marie Luisa, Mulders Daphne GJC, Haagmans Bart L, van der Veer Bas, van den Brink Sharon, Wijsman Lisa, Goderski Gabriel, Romette Jean-Louis, Ellis Joanna, Zambon Maria, Peiris Malik, Goossens Herman, Reusken Chantal, Koopmans Marion PG, Drosten Christian. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25(3):pii=2000045. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045

[2] Email communication between Dr. Peter Borger & Dr. Adam Meijer: Supplementary Material

[3] Jafaar *et al.*, Correlation Between 3790 Quantitative Polymerase Chain Reaction–Positives Samples and Positive Cell Cultures, Including 1941 Severe Acute Respiratory Syndrome Coronavirus 2 Isolates https://academic.oup.com/cid/advance-article/doi/10.1093/cid/ciaa1491/5912603

[4] BBC, January 21st 2020: https://www.bbc.com/news/world-asia-china-51185836; archive: https://archive.is/0qRmZ

[5] Google Analytics - COVID19-deaths worldwide: https://bit.ly/3fndemJ; archive: https://archive.is/PpqEE

[6] Laboratory testing for COVID-19 Emergency Response Technical Centre, NIVD under China CDC March 15th, 2020: http://www.chinacdc.cn/en/COVID19/202003/P020200323390321297894.pdf

[7] Real-Time PCR Handbook Life Technologies (https://www.thermofisher.com/content/dam/LifeTech/global/Forms/PDF/real-time-pcr-handbook.pdf)

Nolan T, Huggett J, Sanchez E.Good practice guide for the application of quantitative PCR (qPCR) First Edition 2013

[8] Trestan Pillonel *et al*, Letter to the editor: SARS-CoV-2 detection by real-time RT-PCR: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7268274/

[9] Kurkela, Satu, and David WG Brown. "Molecular-diagnostic techniques." Medicine 38.10 (2009): 535-540.

[10] Wolfel *et al.,* Virological assessment of hospitalized patients with COVID-2019 https://www.nature.com/articles/s41586-020-2196-x

[11] Thermofischer Primer Dimer Web Tool: https://www.thermofisher.com/us/en/home/brands/thermo-scientific/molecular-biology/molecular-biology-learning-center/molecular-biology-resource-library/thermo-scientific-web-tools/multiple-primer-analyzer.html

[12] Primer-BLAST, NCBI - National Center for Biotechnology Information: https://www.ncbi.nlm.nih.gov/tools/primer-blast/

[13] Marra MA, Steven JMJ, Caroline RA, Robert AH, Angela BW et al. (2003) Science. The Genome sequence of the SARS-associated coronavirus. Science 300(5624): 1399-1404.

[14] Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome: https://www.ncbi.nlm.nih.gov/nuccore/MN908947

[15] Borger P. A SARS-like Coronavirus was expected but nothing was done to be prepared. Am J Biomed Sci Res 2020. https://biomedgrid.com/pdf/AJBSR.MS.ID.001312.pdf https://www.researchgate.net/publication/341120750_A_SARS-like_Coronavirus_was_Expected_but_nothing_was_done_to_be_Prepared; archive: https://archive.is/i76Hu

[16] Eurosurveillance paper evaluation / review process: https://www.eurosurveillance.org/evaluation

[17] Official recommendation of the Corman-Drosten protocol & manuscript by the WHO, published on January 13th 2020 as version 1.0 of the document: https://www.who.int/docs/default-source/coronaviruse/wuhan-virus-assay-v1991527e5122341d99287a1b17c111902.pdf; archive: https://bit.ly/3m3jXVH

[18] Official WHO-recommendation for the Corman / Drosten RT-qPCR-protocol, which directly derives from the Eurosurveillance-publication, document-version 2-1, published on

17th January 2020: https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c_2

[19] Eurosurveillance Editorial Board, 2020: https://www.eurosurveillance.org/upload/site-assets/imgs/2020-09-Editorial%20Board%20PDF.pdf; archive: https://bit.ly/2TqXBjX

[20] Instructions For Use LightMix SarbecoV E-gene plus EAV Control, TIB-Molbiol & Roche Molecular Solutions, January 11th 2020:
https://www.roche-as.es/lm_pdf/MDx_40-0776_96_Sarbeco-E-gene_V200204_09164154001 (1).pdf
Archive, timestamp - January 11th 2020: https://archive.is/Vulo5; archive: https://bit.ly/3fm9bXH

[21] Christian Drosten & Victor Corman, responsible for viral diagnostics at Labor Berlin: https://www.laborberlin.com/fachbereiche/virologie/; archive: archive.is/CDEUG

[22] Tom Jefferson, Elizabeth Spencer, Jon Brassey, Carl Heneghan Viral cultures for COVID-19 infectivity assessment. Systematic review. Systematic review doi: https://doi.org/10.1101/2020.08.04.20167932;
https://www.medrxiv.org/content/10.1101/2020.08.04.20167932v4

[23] Kim *et al.,*The Architecture of SARS-CoV-2 Transcriptome:
https://www.sciencedirect.com/science/article/pii/S0092867420304062

[24] ECDC reply to Dr. Peter Borger, 18th November 2020: Supplementary Material

[25] Prof. Dr. Ulrike Kämmerer & team, survey & Primer-BLAST table: Supplementary Material

Additional literature:

Description RT-PCR RKI Germany, on page 10 of this link:
https://www.rki.de/DE/Content/Gesundheitsmonitoring/Gesundheitsberichterstattung/GBEDownloadsJ/JoHM_S5_2020_Studienprotokoll_CORONA_MONITORING_lokal.pdf?__blob=publicationFile

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Author's Contributions:

PB: Planned and conducted the analyses and research, conceptualising the manuscript.

RKM: Planned and conducted the research, conceptualising the figures and manuscript.

MY: Conducted the analyses and research.

KMcK: Conducted the analyses and research, conceptualized the manuscript.

KS: Conducted the analyses and research.

PMcS: Proofreading the analyses and research.

LA: Proofreading the analyses and research.

FF: Proofreading the analyses and research.

TB: Proofreading the analyses and research.

HU: Proofreading the analyses and research.

MO: Proofreading the analyses and research.

SS: Proofreading the analyses and research.

MDvK: Proofreading the analyses and research.

DG: Proofreading the analyses and research.

RJK: Proofreading the analyses and research.

RS: Proofreading the analyses and research, and the manuscript.

BWK: Proofreading the analyses and research.

RvV: Proofreading the analyses and research.

JB: Proofreading the analyses and research.

KC: Proofreading the analyses and research.

UK: Planned and conducted the analyses and research, conceptualising the manuscript.

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FORM 43A

Rule 43.06(3)

IN THE SUPREME COURT OF VICTORIA AT MELBOURNE
COMMON LAW DIVISON
JUDICIAL REVIEW AND APPEALS LIST

No. S ECI 2021/03031

B E T W E E N

JONATHAN EDWARD KINGSFORD ANDREWS — Plaintiff

-and-

PROFESSOR BRETT SUTTON — First Defendant
(in his capacity as Chief Health Officer as designated under the *COVID Omnibus (Emergency Measures) Act 2020*)

ASSOCIATE PROFESSOR MICHELLE GILES — Second Defendant
(in her capacity as Deputy Health Commander as authorised to exercise emergency powers by Chief Health Officer under section 199(2)9(a) of the *Public Health and Wellbeing Act 2008*)

CERTIFICATE IDENTIFYING EXHIBIT

Date of Document:	24 August 2021	Solicitors Code:	141108
Filed on behalf of:	Jonathan E. K. Andrews	DX:	
Prepared by:	Jonathan E. K. Andrews	Telephone:	(03) 9070 1286
	Bosanquet Solicitors,	Ref:	210801
	TOK Corporate Centre, Level 1	Email:	info@bosanquets.com
	459 Toorak Rd, TOORAK VIC 3142		

This is the exhibit marked "**JA-04**" now produced and shown to JONATHAN EDWARD KINGSFORD ANDREWS at the time of swearing/affirming that person's affidavit on 24 AUGUST 2021:

……

JONATHAN EDWARD KINGSFORD ANDREWS

The Affidavit & Annexures were sworn to and signed by the Deponent by audio visual link and I, as affidavit taker, have used a scanned or electronic copy of the affidavit & annexures and not the original, in completing the jurat requirements under subsection s 27 ss (1) of the Oaths and Affirmations Act 2018 (VIC).

……

Mr Tony Nikolic, Director

Ashley, Francina, Leonard & Associates

Level 25, Tower 3, 300 Barangaroo Avenue,

Sydney, NSW, 2000

(An Australian Legal Practitioner as per Legal Profession Uniform Law [VIC])

Exhibit “JA-04”

World Health Organisation Information Notice for IVD Users 2020/05, Nucleic acid testing (NAT).

World Health Organization

WHO Information Notice for IVD Users 2020/05

Nucleic acid testing (NAT) technologies that use polymerase chain reaction (PCR) for detection of SARS-CoV-2

20 January 2021 | Medical product alert | Geneva | Reading time: 1 min (370 words)

Français Español

Product type: Nucleic acid testing (NAT) technologies that use polymerase chain reaction (PCR) for detection of SARS-CoV-2

Date: 13 January 2021

WHO-identifier: 2020/5, version 2

Target audience: laboratory professionals and users of IVDs.

Purpose of this notice: clarify information previously provided by WHO. This notice supersedes WHO Information Notice for In Vitro Diagnostic Medical Device (IVD) Users 2020/05 version 1, issued 14 December 2020.

Description of the problem: WHO requests users to follow the instructions for use (IFU) when interpreting results for specimens tested using PCR methodology.

Users of IVDs must read and follow the IFU carefully to determine if manual adjustment of the PCR positivity threshold is recommended by the manufacturer.

WHO guidance Diagnostic testing for SARS-CoV-2 states that careful interpretation of weak positive results is needed (*1*). The cycle threshold (Ct) needed to detect virus is inversely proportional to the patient's viral load. Where test results do not correspond with the clinical presentation, a new specimen should be taken and retested using the same or different NAT technology.

WHO reminds IVD users that disease prevalence alters the predictive value of test results; as disease prevalence decreases, the risk of false positive increases (*2*). This means that the probability that a person who has a positive result (SARS-CoV-2 detected) is truly infected with SARS-CoV-2 decreases as prevalence decreases, irrespective of the claimed specificity.

Most PCR assays are indicated as an aid for diagnosis, therefore, health care providers must consider any result in combination with timing of sampling, specimen type, assay specifics, clinical observations, patient history, confirmed status of any contacts, and epidemiological information.

Actions to be taken by IVD users:

1. **Please read carefully the IFU in its entirety.**
2. **Contact your local representative if there is any aspect of the IFU that is unclear to you.**
3. **Check the IFU for each incoming consignment to detect any changes to the IFU.**
4. **Provide the Ct value in the report to the requesting health care provider.**

Contact person for further information:

Anita SANDS, Regulation and Prequalification, World Health Organization, e-mail: rapidalert@who.int

References:

1. Diagnostic testing for SARS-CoV-2. Geneva: World Health Organization; 2020, WHO reference number WHO/2019-nCoV/laboratory/2020.6.

2. Altman DG, Bland JM. Diagnostic tests 2: Predictive values. BMJ. 1994 Jul 9;309(6947):102. doi: 10.1136/bmj.309.6947.102.

FORM 43A

Rule 43.06(3)

IN THE SUPREME COURT OF VICTORIA AT MELBOURNE
COMMON LAW DIVISON
JUDICIAL REVIEW AND APPEALS LIST

No. S ECI 2021/03031

B E T W E E N

JONATHAN EDWARD KINGSFORD ANDREWS — Plaintiff

-and-

PROFESSOR BRETT SUTTON
(in his capacity as Chief Health Officer
as designated under the *COVID Omnibus (Emergency Measures) Act 2020*) — First Defendant

ASSOCIATE PROFESSOR MICHELLE GILES
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the *Public Health and Wellbeing Act 2008*) — Second Defendant

CERTIFICATE IDENTIFYING EXHIBIT

Date of Document:	24 August 2021	Solicitors Code:	141108
Filed on behalf of:	Jonathan E. K. Andrews	DX:	
Prepared by:	Jonathan E. K. Andrews	Telephone:	(03) 9070 1286
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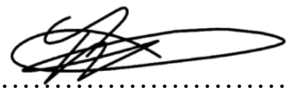
This is the exhibit marked "**JA-05**" now produced and shown to JONATHAN EDWARD KINGSFORD ANDREWS at the time of swearing that person's affidavit on 24 AUGUST 2021:

……

JONATHAN EDWARD KINGSFORD ANDREWS

The Affidavit & Annexures were sworn to and signed by the Deponent by audio visual link and I, as affidavit taker, have used a scanned or electronic copy of the affidavit & annexures and not the original, in completing the jurat requirements under subsection s 27 ss (1) of the Oaths and Affirmations Act 2018 (VIC).



……

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Exhibit "JA-05"

Assessment and Testing Criteria for coronavirus (COVID-19), Victoria Department of Health.

Assessment and testing criteria for coronavirus (COVID-19)

1. The **cycle threshold (Ct)** value of a reaction is the cycle number when the fluorescence of a PCR product is first detected above the background signal. The lower the Ct value, the more virus is present in the sample being tested, as fewer amplification cycles are required before the threshold for detection is met. A high Ct value generally indicates that it takes longer, that is, more cycles to detect the virus, indicating that there is less viral RNA present in the sample. Each PCR assay may have a different Ct value that is used for detecting SARS-CoV-2. Ct values for one in-vitro diagnostic (IVD) device should not be compared with Ct values from other platforms. This means there is no 'set' Ct value to aim for across all platforms. High Ct values are as defined in consultation with the responsible supervising pathologist.

Assessment and testing criteria for coronavirus (COVID-19)

Check who and how to test for COVID-19 and manage cases and contacts.

Victorian coronavirus (COVID-19) testing criteria

9 April 2021

- Fever OR chills in the absence of an alternative diagnosis that explains the clinical presentation*

OR

- Acute respiratory infection (including cough, sore throat, shortness of breath, runny nose)**

OR

- Loss of smell or loss of taste.

*Clinical discretion applies; consider potential for co-infection (such as SARS-CoV-2 and influenza).

**Older people may present with other atypical symptoms including functional decline, delirium, exacerbation of underlying chronic condition, falls, loss of appetite, malaise, nausea, diarrhoea and myalgia.

Other clinical symptoms

People in the following groups should be tested if they have new onset of other clinical symptoms associated with COVID-19 (including headache, myalgia, stuffy nose, nausea, vomiting or diarrhoea):

- people who are most at risk of severe illness
- higher prevalence groups and settings
- settings with a high risk of transmission.

Clinical judgement and reasoning should be used, including consideration of epidemiological risk factors for acquisition and transmission.

Case definitions

Confirmed case

A confirmed case requires laboratory definitive evidence.

Laboratory definitive evidence

- Detection of SARS-CoV-2 by nucleic acid testing[1]

OR

- isolation of SARS-CoV-2 in cell culture, with confirmation using a nucleic acid test

OR

- SARS-CoV-2 IgG seroconversion or a four-fold or greater increase in SARS-CoV-2 antibodies of any immunoglobulin subclass including 'total' assays in acute and convalescent sera, in the absence of vaccination.[2]

Historical case

- A historical case requires laboratory suggestive evidence supported by **either** previous (prior to the past 14 days) **clinical** evidence

OR

- previous (prior to the past 14 days) **epidemiological** evidence.

A historical case should not have any symptoms of COVID-19 (or not have had symptoms of COVID-19 for the past 14 days).

Laboratory suggestive evidence

- Detection of SARS-CoV-2 by polymerase chain reaction (PCR) on two specimens at least 24 hours apart with high Ct values[3] on both specimens AND detection of IgG or total antibody, in the absence of vaccination[2,4];

OR

- Negative PCR result **AND** detection of IgG or total antibody, in the absence of vaccination[2]

OR

- High PCR Ct result on first result, and higher PCR Ct result or negative PCR result on second test, taken >24 hours apart[2]

Clinical evidence

- History of measured (≥37.5°C) or self-reported fever (for example, night sweats, chills)

OR

- History of an acute respiratory infection (for example, cough, shortness of breath, sore throat).
- loss of smell or taste

Epidemiological evidence

In the 14 days prior to illness onset:

- Close contact with a confirmed case
- International travel
- Workers supporting designated COVID-19 quarantine and isolation services
- International border staff
- Air and maritime crew
- Health, aged or residential care workers and staff with potential COVID-19 patient contact
- People who have been in a setting where there is a COVID-19 case
- People who have been in areas with recent local transmission of SARS-CoV-26.

Notes

1. There is possibility for false negative PCR (polymerase chain reaction) results in children, who may mount a brisk immune response resulting in a lower viral load. Public health units may seek serological evidence of SARS-CoV-2 immunity in symptomatic children who are repeatedly PCR negative but are known primary close contacts.
2. Antibody detection must be by a validated assay and included in an external quality assurance program. For all serological responses to be counted as laboratory evidence, a person should not have had a recent history of COVID-19 vaccination.
3. The **cycle threshold (Ct)** value of a reaction is the cycle number when the fluorescence of a PCR product is first detected above the background signal. The lower the Ct value, the more virus is present in the sample being tested, as fewer amplification cycles are required before the threshold for detection is met. A high Ct value

generally indicates that it takes longer, that is, more cycles to detect the virus, indicating that there is less viral RNA present in the sample. Each PCR assay may have a different Ct value that is used for detecting SARS-CoV-2. Ct values for one in-vitro diagnostic (IVD) device should not be compared with Ct values from other platforms. This means there is no 'set' Ct value to aim for across all platforms. High Ct values are as defined in consultation with the responsible supervising pathologist.

4. Occasionally a person may have a positive PCR result, with high Ct values, on a subset of gene targets in the PCR assay/s used. These scenarios might be due to acute infection but could also represent previous infection (that is, intermittent/persistent SARS-CoV-2 shedding in a historical case), sometimes with a concurrent upper respiratory tract infection due to another organism. A further swab collected at least 24 hours after the positive sample and serology testing can assist in distinguishing an acute from a historical COVID-19 infection. If the person is symptomatic, a full respiratory panel for other pathogens should be done.

Case notification

Both confirmed and historical cases need to be notified to the department.

You should notify the department of confirmed and historical cases as soon as practicable by either:

- using the online COVID-19 notification form (https://forms.business.gov.au/smartforms/servlet/SmartForm.html?formCode=novelcoronavirus) or
- calling 1300 651 160, 24 hours a day.

People who have previously been diagnosed and managed overseas or in another Australian jurisdiction do not need to be notified as a confirmed or historical case.

On this page

Testing criteria

- Who should be tested for coronavirus (COVID-19)?
- Other clinical symptoms
- Who should not be tested for coronavirus (COVID-19)?
- What are the recommendations for testing children?
- What are the recommendations for testing aged care residents?
- Why can't we test everyone for coronavirus (COVID-19)?
- How is coronavirus excluded in symptomatic patients?
- What about hay fever and asthma?

Assessment and procedures

- How do I assess a patient with respiratory symptoms in a community setting?
- How do I perform a coronavirus (COVID-19) swab?
- Do patients need to isolate whilst waiting for test results?
- Where are coronavirus (COVID-19) test locations?

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- PCR-based tests
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Notifying patients

- When do I need to notify a case to the department?
- I am a general practitioner and have ordered a coronavirus (COVID-19) test – who will notify the patient of the results?
- I am a clinician in a health service and have ordered a coronavirus (COVID-19) test – who will notify the patient of the results?
- Why is the department also contacting patients with a positive coronavirus (COVID-19) test result?

Further resources

- Factsheets for patients with coronavirus (COVID-19) and close contacts
- Where can I find more information about coronavirus (COVID-19)?

Key resources

This page is based off the current Victorian guidelines. Please ensure you refer to the latest version of these documents as guidance may change.

- Case and contact management guidelines for health services and general practitioners – 15 April 2021 (PDF) (/coronavirus-case-and-contact-management-guidelines-health-services-and-general-practitioners-covid-19-pdf)
- Case and contact management guidelines for health services and general practitioners – 15 April 2021 (Word) (/coronavirus-case-and-contact-management-guidelines-health-services-and-general-practitioners)

This resource is also listed in the popular resources section on the For health services and professionals - coronavirus (COVID-19) page (health-services-and-professionals-coronavirus-covid-19)

- General practice quick reference guide – Version 25 - 22 February 2021 (Word) (/coronavirus-disease-2019-covid-19-general-practice-quick-reference-guide-doc)
- General practice quick reference guide – Version 25 - 22 February 2021 (PDF) (/coronavirus-disease-2019-covid-19-general-practice-quick-reference-guide-pdf)

Testing criteria

Who should be tested for coronavirus (COVID-19)?

Test all cases meeting the current criteria for coronavirus (COVID-19) testing:

- Fever OR chills in the absence of an alternative diagnosis that explains the clinical presentation*

OR

- Acute respiratory infection (including cough, sore throat, shortness of breath, runny nose)**

OR

- Loss of smell or loss of taste.

*Clinical discretion applies; consider potential for co-infection (such as SARS-CoV-2 and influenza).

**Older people may present with other atypical symptoms including functional decline, delirium, exacerbation of underlying chronic condition, falls, loss of appetite, malaise, nausea, diarrhoea and myalgia.

Other clinical symptoms

People in the following groups should be tested if they have new onset of other clinical symptoms associated with COVID-19 (including headache, myalgia, stuffy nose, nausea, vomiting or diarrhoea):

- people who are most at risk of severe illness
- higher prevalence groups and settings
- settings with a high risk of transmission.

Clinical judgement and reasoning should be used, including consideration of epidemiological risk factors for acquisition and transmission.

Who should not be tested for coronavirus (COVID-19)?

Patients without symptoms should not be tested except in special circumstances where requested by the department, such as:

- as part of an outbreak investigation/response (active case finding)
- all primary close contacts and returned international travellers at the start and the end of quarantine as directed by the department.
- prior to surgery as directed by the department
- as part of department-led enhanced surveillance to:
    - investigate how widespread COVID-19 is in the community, or
    - detect and reduce transmission, particularly in higher prevalence groups and settings and settings with a high risk of transmission.

What are the recommendations for testing children?

- The same testing criteria applies to adults and children of all ages.
- For advice on testing technique in children refer to the Royal Children's COVID-19 swabbing (https://www.rch.org.au/clinicalguide/guideline_index/COVID-19_swabbing/)
- Where possible, family members (parents/carers and children) who meet the testing criteria should be tested at the one location. For advice on which testing sites children can attend for testing refer to the getting tested (/getting-tested)
- Asymptomatic parents/carers are not required to self-isolate whilst their child is awaiting their swab results, unless the Department of Health has told them to. Reasons to self-isolate may include if they are primary or secondary close contacts of a confirmed case of coronavirus (COVID-19).

What are the recommendations for testing aged care residents?

Please refer to advice on the Aged Care Sector coronavirus (COVID-19) (/aged-care-sector-coronavirus-disease-covid-19) page.

Why can't we test everyone for coronavirus (COVID-19)?

All symptomatic patients should be tested for coronavirus (COVID-19) – current testing criteria are found at Assessment and testing for coronavirus (COVID-19) (/assessment-and-testing-criteria-coronavirus-covid-19). Testing criteria will continue to be updated as more is known about the disease and the risk factors for infection.

People without symptoms should not be tested except in special circumstances (/assessment-and-testing-criteria-coronavirus-covid-19).

If a person without symptoms is tested and the result is negative, it does not mean that they have not been infected, as they might still be incubating the virus (See also: "What is the incubation period? (/about-coronavirus-covid-19-

health-professionals)"). In other words, a negative test in an asymptomatic person does not necessarily rule out coronavirus (COVID-19) infection.

How is coronavirus excluded in symptomatic patients?

For patients with fever or respiratory tract infection who are not hospitalised and who do not have an epidemiological link to a known coronavirus (COVID-19) case, a single negative oropharyngeal and deep nasal swab (plus sputum if possible) is sufficient to exclude coronavirus (COVID-19) infection.

Repeat testing (especially of lower respiratory tract specimens) in clinically compatible cases should be performed if initial results are negative and there remains a high index of suspicion of infection. In unwell patients, consideration should also be given to a respiratory virus panel test, especially if the first coronavirus (COVID-19) test is negative.

There is no strong evidence to support a required time interval between exclusion swabs or the need for multiple practitioners performing the swab. Medical practitioners should apply discretion and consider the need for this on a case by case basis.

What about hay fever and asthma?

Clinical judgement should be used to decide whether rhinorrhoea represents possible coronavirus (COVID-19) (requiring testing) or allergic rhinitis. There should be a low threshold for testing for coronavirus (COVID-19), particularly if the symptoms are different to the patient's usual hay fever or asthma symptoms.

Factors that would make allergic rhinitis more likely include:

- history of allergic rhinitis in previous years at a similar time of year
- concomitant itchy nose and eyes
- response to usual treatments

Factors that would make coronavirus (COVID-19) more likely include:

- other respiratory symptoms (sore throat, cough, anosmia) or
- systemic symptoms (such as fever, myalgia, anorexia)

- no or minimal response to usual treatments.

People with known allergic rhinitis or asthma should have:

- their treatment optimised, including through having an up-to-date asthma action plan or hay fever treatment plan.
- advice about what symptoms might suggest COVID-19 infection and require them to immediately get tested for COVID-19 and stay home until a negative result is received.

Assessment and procedures

How do I assess a patient with respiratory symptoms in a community setting?

- Separate from other patients.
- Place single-use surgical mask on the patient.
- Use droplet and contact precautions (gown, gloves, eye protection and single-use face mask) when assessing the patient.
- Conduct a medical assessment, and focus on:
    - date of onset of illness and especially whether there are symptoms or signs of pneumonia
    - contact with confirmed or historical cases (/assessment-and-testing-criteria-coronavirus-covid-19) of COVID-19
    - precise travel history and occupation
    - history of contact with sick people, travellers, or healthcare facilities
    - work or residence in higher prevalence groups and settings, settings with a high risk of transmission or other priority settings and groups
    - co-morbidities – see People who are most at risk of severe illness (/clinical-guidance-and-resources-covid-19).

All patients should attend an emergency department if clinical deterioration occurs. If clinically required, ambulance transport should be used - advise 000 operator of suspected coronavirus (COVID-19).

Further information

- General practice quick guide (/coronavirus-disease-2019-covid-19-general-practice-quick-reference-guide-doc)
- Infection control guidelines (/covid-19-infection-control-guidelines)

How do I perform a coronavirus (COVID-19) swab?

If you have a patient who meets the criteria for coronavirus (COVID-19) testing (and who does not have symptoms or signs of pneumonia):

- Place a surgical mask on the patient and isolate them in a single room with door closed.
- Use droplet and contact precautions (single-use surgical face mask, eye protection, gown and gloves).
- Collect specimens for coronavirus (COVID-19) testing:
    - Take a single oropharyngeal and deep nasal swab for coronavirus (COVID-19) PCR. To conserve swabs the same swab that has been used to sample the oropharynx should be utilised for deep nosesampling (i.e. one swab per patient only).
    - Oropharyngeal (throat): swab the tonsillar beds and the back of the throat, avoiding the tongue.
    - Deep nasal:
        - Using a pencil grip and while gently rotating the swab, insert the tip 2-3 cm (or until resistance is met) into the nostril, parallel to the palate, to absorb mucoid secretion.
        - Rotate the swab several times against the nasal wall.
        - Withdraw the swab and repeat the process in the other nostril. To conserve swabs, the same swab that has been used to sample the oropharynx should be utilised for nasal sampling.
- Place the swab back into the accompanying transport medium.
- Take blood in a serum tube for storage at VIDRL.
- Consider alternative causes, in particular consider testing for other respiratory viruses using a multiplex PCR if available. Ask your local

laboratory if they require a second swab for this.

If appropriate personal protective equipment is unavailable, direct the patient to the nearest coronavirus (COVID-19) assessment centre.

Patients with symptoms and signs suggestive of pneumonia should be tested and treated in hospital.

For advice on testing technique in children refer to the Royal Children's COVID-19 swabbing (https://www.rch.org.au/clinicalguide/guideline_index/COVID-19_swabbing/) page.

Do patients need to isolate whilst waiting for test results?

People who were **symptomatic** at the time of testing for COVID-19 should isolate until COVID-19 is excluded, **unless advised to isolate for longer by the department**. If their test is negative, they should continue to isolate until the acute symptoms have resolved and they feel well.

People who were **asymptomatic** at the time of testing for COVID-19 should isolate until COVID-19 is excluded, **unless advised otherwise by the department.** Those advised by the department that they do not need to quarantine include:

- those tested as part of a surveillance testing or other targeted testing program **and**
- who are not being tested due to concerns about cases that might have been missed.

Where are coronavirus (COVID-19) test locations?

This map provides GPs and primary care practitioners with details of local Coronavirus (COVID-19) GP Respiratory Clinics and Hospital Respiratory Clinics for referral purposes: GP Respiratory Clinics and Hospital Respiratory Clinics (COVID-19) (/gp-respiratory-clinics-and-hospital-respiratory-clinics-covid-19)

What testing is currently available for coronavirus (COVID-19)?

PCR-based tests

Molecular testing on a well-collected single throat and deep nasal swab is the current test of choice for the diagnosis of acute coronavirus (COVID-19) infection. Molecular tests use real-time polymerase chain reaction (PCR) to look for evidence of the genetic material (RNA) of SARS-CoV-2 (the virus that causes coronavirus COVID-19). Because these tests directly detect viral RNA, they are an indicator for viral shedding.

A positive PCR result indicates current or very recent infection. SARS-CoV-2 RNA is generally detectable in respiratory specimens from about one day prior to symptom onset, and during the acute phase of infection. Patients may continue to shed viral RNA after their symptoms resolve, but the extent to which this correlates with transmissibility is currently unclear. Clinical resolution (and for some cases two consecutive negative PCR tests) are currently being used as criteria when considering release from isolation. However, this may change as our knowledge of the virus increases.

A negative PCR result means that SARS-CoV-2 RNA was not identified in the sample. Negative results do not preclude SARS-CoV-2 infection, and interpretation of such results should be combined with clinical observations, patient history, and epidemiological information.

Serology-based tests

There are several serology tests currently undergoing assessment in Australia but the accuracy and clinical utility of these have not yet been established. In the interim, clinicians assessing patients with suspected coronavirus (COVID-19) infection can send serum to VIDRL (the state reference laboratory) for storage so that serology can be performed once a test becomes available.

Serology-based tests detect antibodies that develop in response to coronavirus (COVID-19) infection. Early reports indicate that it may take seven days or more from the time a patient first becomes sick for antibodies to be detectable. Therefore, these tests are of limited use for the diagnosis of acute infection. Elderly or immunocompromised patients may never (or only much later) develop antibodies to the virus that causes coronavirus (COVID-19), and

therefore may return a negative test despite infection. Because antibody tests do not detect active viral shedding, they cannot detect if an individual is infectious.

Several serology-based point-of-care tests (PoCT) for coronavirus (COVID-19) have recently been approved by the Therapeutic Goods Administration (TGA) subject to conditions. The conditions require that additional evidence to support the ongoing safety and performance of these tests be provided to the TGA within 12 months of approval. These PoCTs are yet to be validated in Australia and are not currently recommended for the acute diagnosis of coronavirus (COVID-19) infection as they will miss patients in the early stages of the disease when they are infectious to other people.

For more information please see Communique: Point of Care Testing for COVID-19 (Word) (/communique-point-care-testing-covid-19).

See the position statements by the Royal College of Pathologists of Australasia and the Public Health Laboratory Network on PoCT for coronavirus (COVID-19) on the Royal College of Pathologists website (https://www.rcpa.edu.au/Library/COVID-19-Updates/COVID-19-Useful-Resources). Further information is also available on the TGA website (https://www.tga.gov.au/covid-19-testing-australia-information-health-professionals).

Notifying patients

When do I need to notify a case to the department?

You should notify the department of confirmed cases as soon as possible by either:

- using the online COVID-19 notification form (https://forms.business.gov.au/smartforms/servlet/SmartForm.html?formCode=novelcoronavirus) or
- calling 1300 651 160, 24 hours a day.

Notification to the department is only required for confirmed and historical cases.

I am a general practitioner and have ordered a coronavirus (COVID-19) test – who will notify the patient of the results?

It is the responsibility of the general practitioner who ordered the coronavirus (COVID-19) test to ensure arrangements are in place for contacting the patient with the test result, regardless of whether it is negative or positive.

If the result is positive, notify the Department of Health by either calling 1300 651 160 or using the online COVID-19 notification form. (https://forms.business.gov.au/smartforms/servlet/SmartForm.html?formCode=novelcoronavirus)

All confirmed cases of coronavirus (COVID-19) are legally required to isolate (https://www.coronavirus.vic.gov.au/what-do-if-you-have-coronavirus-covid-19) until they are cleared by the Department of Health or their nominated representative.

I am a clinician in a health service and have ordered a coronavirus (COVID-19) test – who will notify the patient of the results?

It is the responsibility of the testing clinician and health service who ordered the coronavirus (COVID-19) test to ensure arrangements are in place for contacting the patient with the test result, regardless of whether it is negative or positive.

If the result is positive, the health service infectious diseases lead, or senior clinician should notify the Department of Health (including additional clinical information) by either:

- using the **online COVID-19 notification form** (https://forms.business.gov.au/smartforms/servlet/SmartForm.html?formCode=novelcoronavirus), or
- calling **1300 651 160**, 24 hours a day.

All confirmed cases of coronavirus (COVID-19) are legally required to isolate (https://www.coronavirus.vic.gov.au/what-do-if-you-have-coronavirus-covid-19) until they are cleared by the Department of Health or their nominated representative.

Why is the department also contacting patients with a positive coronavirus (COVID-19) test result?

The department receives notification from laboratories of all positive results. The department contacts all confirmed cases (people who test positive) to conduct an interview, provide information about self-isolation and trace close contacts. To prevent administrative hold-ups in the investigation process, the department is no longer waiting for the treating doctor to inform the patient of their result before making contact.

Even if the department has already contacted your patient with a positive coronavirus (COVID-19) test result, the treating doctor or clinical team representative (as appropriate) should still contact the patient. This is important to ensure that:

- the patient has received their result
- any clinical queries have been addressed
- there is a clear management plan in place.

Call the department on **1300 651 160** to provide any additional clinical information and/or agree on next steps for management of the patient.

All confirmed cases of coronavirus (COVID-19) must isolate (https://www.coronavirus.vic.gov.au/what-do-if-you-have-coronavirus-covid-19) until they are cleared by the Department of Health or their nominated representative.

Further resources

Factsheets for patients with coronavirus (COVID-19) and close contacts

- Coronavirus (COVID-19) confirmed case - what you need to know (Word) (/novel-coronavirus-confirmed-case-what-you-need-know)
- Factsheet – suspected case (Word) (/novel-coronavirus-suspected-case-what-you-need-know-doc)

FORM 43A

Rule 43.06(3)

IN THE SUPREME COURT OF VICTORIA AT MELBOURNE
COMMON LAW DIVISON
JUDICIAL REVIEW AND APPEALS LIST

No. S ECI 2021/03031

B E T W E E N

JONATHAN EDWARD KINGSFORD ANDREWS — Plaintiff

-and-

PROFESSOR BRETT SUTTON
(in his capacity as Chief Health Officer
as designated under the *COVID Omnibus (Emergency Measures) Act 2020*) — First Defendant

ASSOCIATE PROFESSOR MICHELLE GILES
(in her capacity as Deputy Health Commander
as authorised to exercise emergency powers by
Chief Health Officer under section 199(2)9(a) of
the *Public Health and Wellbeing Act 2008*) — Second Defendant

CERTIFICATE IDENTIFYING EXHIBIT

Date of Document:	24 August 2021	Solicitors Code:	141108
Filed on behalf of:	Jonathan E. K. Andrews	DX:	
Prepared by:	Jonathan E. K. Andrews	Telephone:	(03) 9070 1286
	Bosanquet Solicitors,	Ref:	210801
	TOK Corporate Centre, Level 1	Email:	info@bosanquets.com
	459 Toorak Rd, TOORAK VIC 3142		

This is the exhibit marked "**JA-06**" now produced and shown to JONATHAN EDWARD KINGSFORD ANDREWS at the time of swearing that person's affidavit on 24 AUGUST 2021:

……

JONATHAN EDWARD KINGSFORD ANDREWS

The Affidavit & Annexures were sworn to and signed by the Deponent by audio visual link and I, as affidavit taker, have used a scanned or electronic copy of the affidavit & annexures and not the original, in completing the jurat requirements under subsection s 27 ss (1) of the Oaths and Affirmations Act 2018 (VIC).

……

Mr Tony Nikolic, Director

Ashley, Francina, Leonard & Associates

Level 25, Tower 3, 300 Barangaroo Avenue,

Sydney, NSW, 2000

(An Australian Legal Practitioner as per Legal Profession Uniform Law [VIC])

Exhibit "JA-06"

'Isolation and rapid sharing of the 2019 novel coronavirus (SARS-CoV-2) from the first patient diagnosed with COVID-19 in Australia' The Medical Journal of Australia.

Isolation and rapid sharing of the 2019 novel coronavirus (SARS-CoV-2) from the first patient diagnosed with COVID-19 in Australia

Leon Caly[1], Julian Druce[1], Jason Roberts[1], Katherine Bond[1], Thomas Tran[1], Renata Kostecki[1], Yano Yoga[1], William Naughton[2], George Taiaroa[3], Torsten Seemann[5], Mark B Schultz[5], Benjamin P Howden[5], Tony M Korman[2], Sharon R Lewin[3,4], Deborah A Williamson[5,6], Mike G Catton[1]

The known: By 12 March 2020, 140 cases of COVID-19 (the illness caused by SARS-CoV-2) had been confirmed in Australia; three patients had died. At the end of January, the sequence of the virus had been shared but no laboratory outside China had grown the virus or had access to live virus.

The new: We describe the clinical course and laboratory features of the first reported case of COVID-19 in Australia, as well as the isolation, sequencing, imaging, and rapid global sharing of virus isolated from the patient.

The implications: Rapid identification, propagation and international sharing of SARS-CoV-2 is an important step in collaborative scientific efforts and diagnostic test validation in response to this public health emergency.

Abstract

Objectives: To describe the first isolation and sequencing of SARS-CoV-2 in Australia and rapid sharing of the isolate.

Setting: SARS-CoV-2 was isolated from a 58-year-old man from Wuhan, China who arrived in Melbourne on 19 January 2020 and was admitted to the Monash Medical Centre, Melbourne from the emergency department on 24 January 2020 with fever, cough, and progressive dyspnoea.

Major outcomes: Clinical course and laboratory features of the first reported case of COVID-19 (the illness caused by SARS-CoV-2) in Australia; isolation, whole genome sequencing, imaging, and rapid sharing of virus from the patient.

Results: A nasopharyngeal swab and sputum collected when the patient presented to hospital were each positive for SARS-CoV-2 (reverse transcription polymerase chain reaction). Inoculation of Vero/hSLAM cells with material from the nasopharyngeal swab led to the isolation of SARS-CoV-2 virus in culture. Electron microscopy of the supernatant confirmed the presence of virus particles with morphology characteristic of viruses of the family *Coronaviridae*. Whole genome sequencing of the viral isolate and phylogenetic analysis indicated the isolate exhibited greater than 99.99% sequence identity with other publicly available SARS-CoV-2 genomes. Within 24 hours of isolation, the first Australian SARS-CoV-2 isolate was shared with local and overseas reference laboratories and major North American and European culture collections.

Conclusions: The ability to rapidly identify, propagate, and internationally share our SARS-CoV-2 isolate is an important step in collaborative scientific efforts to deal effectively with this international public health emergency by developing better diagnostic procedures, vaccine candidates, and antiviral agents.

The recognition in 2019 of the first outbreak in Wuhan, China, of a respiratory disease (COVID-19) associated with a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) triggered an international response unparalleled in its scale and tempo.[1–6] In particular, the rapid sharing and integration of clinical and epidemiological data has facilitated understanding of the spectrum of clinical disease caused by SARS-CoV-2 and the extent of its global spread, although there are still many unanswered questions. Further, rapid genomic analyses have corroborated epidemiological investigations, suggesting a global point source outbreak of a novel betacoronavirus originating in Wuhan.[2,5]

The fundamental pillars of the control of any infectious disease are effective prevention, diagnostic, and treatment strategies. For viral pathogens, the propagation of live virus and the timely dissemination of the viral isolate to domestic and international scientific and public health agencies are critical. Rapid sharing of material has allowed laboratories to validate their diagnostic assays and to confirm their ability to detect SARS-CoV-2. In this report we describe the clinical course and laboratory features of the first reported case of COVID-19 in Australia, together with the isolation, sequencing, and imaging of the virus.

Case report and clinical course

A 58-year-old man from Wuhan, China, felt unwell on the day of his arrival in Melbourne (19 January 2020). In China, he had had no contact with live food markets, people known to have COVID-19, or hospitals. His medical history included type 2 diabetes mellitus, and he had ceased smoking four years previously. He developed fever on 20 January and a cough with sputum production on 23 January; on 24 January, he was admitted to the Monash Medical Centre, Melbourne, from its emergency department with progressive dyspnoea. His temperature was 38.1°C, his heart rate 95 beats/min, and O_2 saturation 94% on room air. A chest x-ray showed subtle ill-defined opacities in the middle zones bilaterally and in the left lower zone. A thoracic computed tomography scan on admission day four identified extensive ground glass opacities with a peribronchovascular and peripheral distribution in the middle to upper zones of the lungs (Box 1). Full blood examination results included a lymphocyte count of 0.80×10^9/L (reference range, $1.0–4.0 \times 10^9$/L). C-reactive protein concentration peaked on admission day 6 at 182 mg/L (reference range, 0–5 mg/L). Liver function test abnormalities peaked on admission day 12 — alkaline phosphatase, 210 U/L (reference range, 30–110 U/L); γ-glutamyltransferase, 416 U/L (reference range, 30–110 U/L); alanine aminotransferase, 183 U/L (reference range, 5–40 U/L) — and hepatic steatosis was evident on liver ultrasound. Intravenous ceftriaxone (2 g/day)

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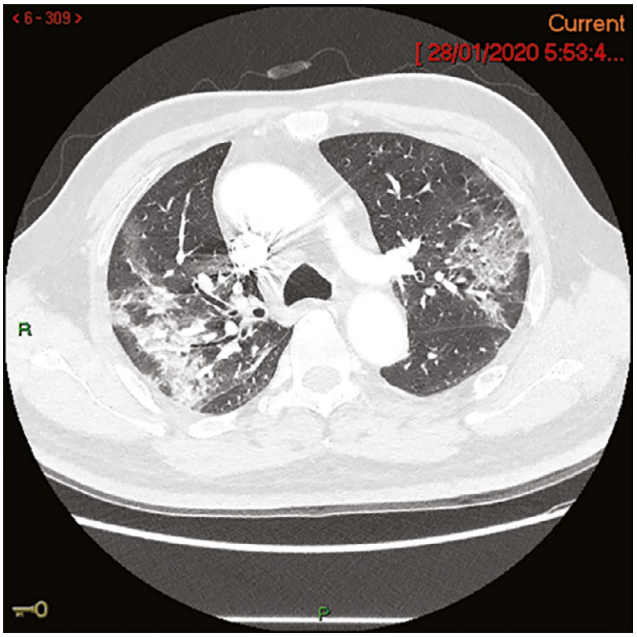
sharon.lewin@unimelb.edu.au
• doi: 10.5694/mja2.50569 • The unedited version of this article was published as a preprint mja.com.au on 9 March 2020 • See Editorial (Smith).

1 Thoracic computed tomography (CT) image of patient on admission day 4



and azithromycin (500 mg/day) were commenced on admission day 4 to treat potential secondary bacterial pneumonia, although no bacterial pathogen was identified. Low-flow oxygen (maximum 3 L/min via nasal prongs) was administered until admission day 10. The patient gradually improved; fever, productive cough and dyspnoea resolved by admission day 12, and he was discharged from hospital on 7 February (admission day 15).

Methods

Diagnostic testing for SARS-CoV-2

Real time reverse transcription (RT) polymerase chain reaction (PCR) testing for SARS-CoV-2 was performed on material from an initial nasopharyngeal swab in 200 µL viral transport medium, and separately for sputum, urine, faeces, and serum samples. Briefly, an in-house real time RT-PCR assay was developed, and all positive tests confirmed by nested RT-PCR, using previously described methods.[7] PCR products underwent in-house Sanger sequencing, which confirmed the presence of SARS-CoV-2 (online Supporting Information, 1.1–1.3).

Virus culture and electron microscopy

Material from the initial nasopharyngeal swab was used to inoculate a Vero/hSLAM cell line (European Collection of Authenticated Cell Cultures [ECACC] #04091501). Flasks were monitored for the development of viral cytopathic effect and 140 µL aliquots removed every 48 hours to assess virus burden by real time RT-PCR.

For electron microscopy, a 4 mL aliquot of supernatant from cell cultures grown in the presence of 4 µg/mL trypsin was inactivated with 0.5% glutaraldehyde for 12 h and clarified by centrifugation at 1000 g for 3 min. Supernatant was negatively stained with 3% phosphotungstic acid (pH 7.0) and examined with an FEI Tecnai T12 electron microscope at 80kV. The remaining pellet was stained *en bloc* and embedded in resin; 70 nm sections were examined with an FEI Tecnai F30 electron microscope at 200kV (Supporting Information, 2.1–2.2).

Whole genome sequencing of SARS-CoV-2 and bioinformatic analysis

We extracted RNA for whole genome sequencing of the viral isolate. Briefly, RNA was extracted from clarified cell culture supernatant and randomly amplified cDNA prepared by sequence-independent single-primer amplification (SISPA).[8,9] Sequencing was performed with a combination of Oxford Nanopore Technologies and Illumina short-read sequencing. Genomic assembly of the BetaCoV/Australia/VIC/01/2020 genome was confirmed by parallel *de novo* and reference-guided methods (Supporting Information, 3.1–3.4).

Results

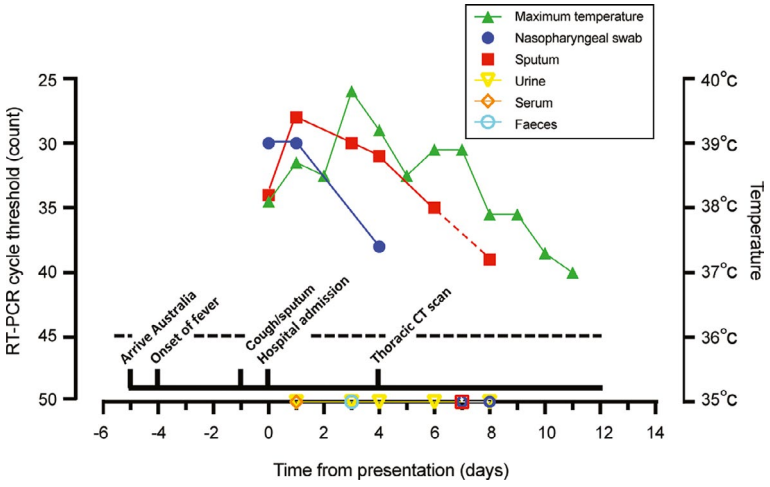
Detection of SARS-CoV-2 in clinical samples

A nasopharyngeal swab and sputum collected on presentation were positive for SARS-CoV-2 on real time RT-PCR assay. Serial daily RT-PCR testing of nasopharyngeal swabs and sputum from the patient indicated a gradual decline in viral load in sputum between admission days 1 and 8, and a decline in viral load and disappearance from nasopharyngeal swabs by admission day 7. No virus was detected in urine samples, nor in single faecal (admission day 3) or plasma samples (admission day 1) (Box 2).

Growth, visualisation, and global sharing of SARS-CoV-2 virus

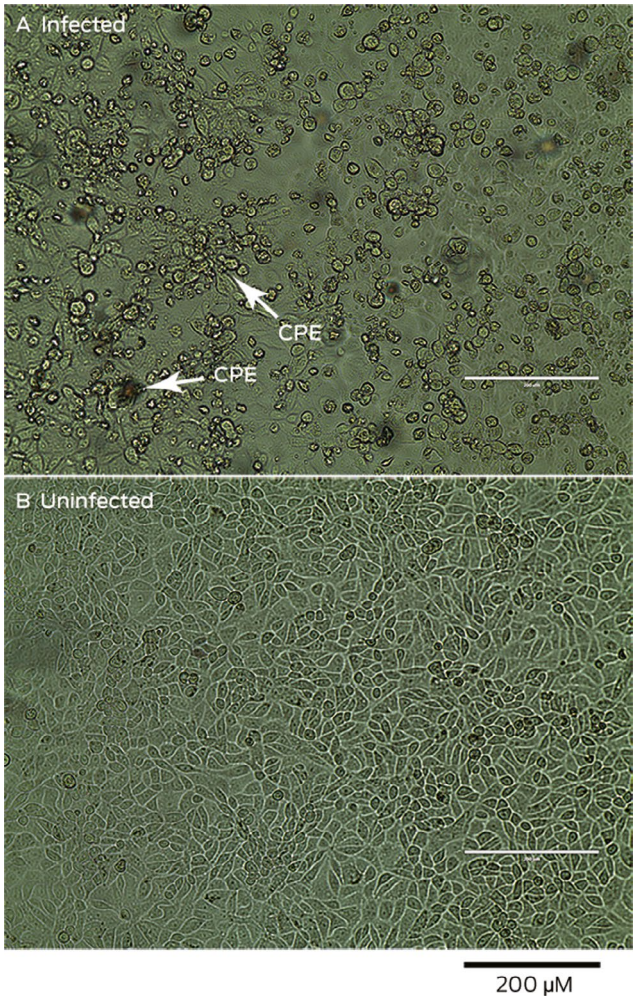
Two days after inoculation of the VERO/hSLAM cell line, a subtle viral cytopathic effect was observed, and was distinct at day 6 compared with an uninfected control cell line (Box 3). RT-PCR testing of the cell line supernatant confirmed a high viral load, suggesting productive viral infection (Box 4). Electron micrographs of the negatively stained supernatant showed spherical

2 Clinical course of first Australian case of COVID-19, including laboratory investigations

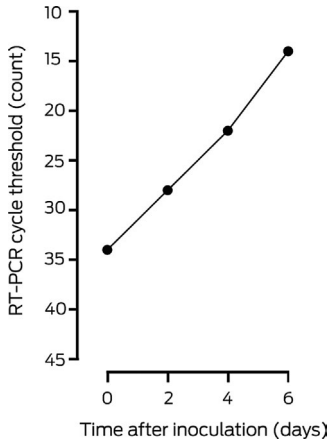


SARS-CoV-2 was quantified by real time RT-PCR. The cycle threshold count is shown for each specimen type; an increase in count value is consistent with reduced viral load. The assay limit of detection (dashed line) threshold is a count of 45; open symbols beneath the threshold indicate null detection of virus. ◆

3 Light microscopy of Vero/hSLAM cells. A. Cells infected with material from patient, with viral cytopathic effect (CPE) evident six days after inoculation. B. Uninfected (control) cells



4 SARS-CoV-2 viral load, quantified by real time polymerase chain reaction (RT-PCR) of supernatant from virus-infected Vero/hSLAM cell culture*



* Lower threshold cycle count values indicate higher viral loads. ◆

and pleomorphic virus-like particles of 90–110 nm diameter; the particles displayed prominent spikes (9–12 nm), characteristic of viruses from the family *Coronaviridae* (Box 5, A). Electron micrographs of sectioned VERO/hSLAM cells showed cytoplasmic membrane-bound vesicles containing coronavirus particles (Box 5, B) Following several failures to recover virions with the characteristic fringe of surface spike proteins, it was found that adding trypsin to the cell culture medium immediately improved virion morphology.

In consultation with the World Health Organization, the viral isolate was shared with domestic and international reference laboratories within 24 hours, and lodgement with major North American and European culture collections for further distribution is underway.

Phylogenetic analysis

Phylogenetic analysis indicated that the genome sequence of our isolate (BetaCoV/Australia/VIC01/2020) exhibited greater than 99.99% sequence identity with other publicly available SARS-CoV-2 genomes (online Supporting Information, 3.4), consistent with the epidemiological features of this case originating in Wuhan.[10] Compared with the National Center for Biotechnology Information (NCBI) SARS-CoV-2 reference sequence (NC_045512.3), there were three previously described single nucleotide polymorphisms and a 10 base pair deletion in the 3′ untranslated region (3′UTR) (Supporting Information, 3.4). Our sequences are available at GenBank (accession number, MT007544.1), and the genome was rapidly uploaded to the Global Initiative of Sharing All Influenza (GISAID) (accession number, EPI_ISL_406844).

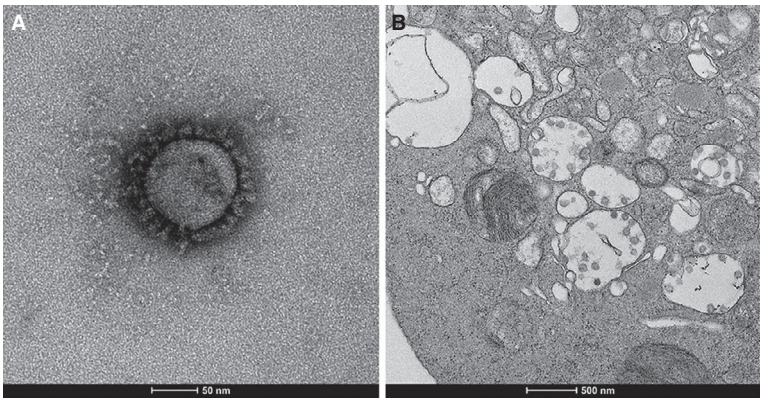
Discussion

We have described the first reported case of COVID-19 in Australia, with rapid diagnosis, and isolation, imaging, and sharing of the causative agent, SARS-CoV-2. By 12 March 2020, there had been 140 confirmed cases in Australia; three patients had died.[11] Although 65% of confirmed cases around the world have been reported from China, an increasing number are being reported in South Korea, Italy, and Iran, and limited human-to-human transmission has been described.[6,10–12] Although the number of cases in Australia is relatively small, the political and societal effects (as in other countries) have already been considerable, including travel restrictions to and from mainland China, the Republic of Korea, Italy, and Iran.[13] The sustainability of these measures and their effects on local and global control remain to be established, but the consequences of the outbreak will probably be felt for many months, if not longer.

The clinical features in our case were consistent with other recent reports, including the initial presentation of fever, cough, and progressive dyspnoea.[3,6] It is notable that the viral burden was greatest in sputum specimens, which remained positive for SARS-CoV-2 for eight days after initial presentation, compared with four days for nasopharyngeal swabs (Box 2). The decline in viral load was correlated with the resolution of fever and, ultimately, clinical improvement. One unresolved question is whether patients who are clinically stable and deemed fit to be discharged from hospital but have PCR-detectable virus are infectious, or whether this indicates only the persistence of non-infectious, residual viral RNA.

We applied standard techniques to isolate the virus, but we were the first group to isolate it outside China during the early stages of the epidemic. Potential reasons for our success could be the

5 Electron micrographs of cell culture supernatant. A. 100 nm spherical virion displaying the characteristic crown-like fringe of spike proteins. B. Infected VERO/hSLAM sections with membrane-bound vesicles containing virus



viral burden of the collected specimens and the extensive clinical experience in our reference laboratory.

An important aspect of the scientific response to the COVID-19 outbreak has been the rapid sharing of information about diagnostic assays and genomic data, enabling rapid elucidation of the emergence and spread of the novel virus. In addition, a major principle of our laboratory response in Australia was to immediately share the viral isolate with the WHO and other laboratories to facilitate rapid validation of diagnostic testing. We continue to share live virus with other agencies, both locally and overseas, involved in the development and testing of therapeutic agents and vaccines. This is an essential function of public health reference and research laboratories, and we strongly encourage others to apply a similarly collaborative approach to streamlining efforts to diagnose, prevent, and treat COVID-19 during this public health emergency.

Acknowledgements: Sharon Lewin receives research support from the National Health and Medical Research Council (NHMRC). Sharon Lewin and Benjamin Howden are NHMRC Practitioner Fellows; Deborah Williamson holds an NHMRC Investigator grant. Sharon Lewin and Mike Catton are supported by the Australian Partnership for Preparedness Research in Infectious Diseases Emergencies (APPRISE), an NHMRC-funded Centre for Research Excellence.

We acknowledge our public health partners and the Victorian Department of Health and Human Services, the major funder of the Victorian Infectious Diseases Reference Laboratory, without whom this work would not have been possible. We also thank the clinical and laboratory staff involved in the care of our patient. We thank Andrew Leis and Eric Hanssen (Melbourne Advanced Microscopy Facility, Bio21 Institute) for their assistance with the FEI L120C (during preliminary screening) and FEI F30 microscopes, and Susan Ballard and Michelle Sait (Microbiological Diagnostic Unit Public Health Laboratory, University of Melbourne) for assistance with Illumina sequencing. We also thank Dave O'Connor (University of Wisconsin Medical Foundation) for his support and assistance in establishing the SISPA protocol at the Doherty Institute. We thank Jasminka Sterjovski for assisting with preparation and submission of the manuscript.

Competing interests: No relevant disclosures. ■

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1 Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. *Nature* 2020; 579: 265–269.

2 Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020; 579: 270–273.

3 Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; 395: 497–506.

4 Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. *Lancet* 2020; 395: 470–473.

5 Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* 2020; 382: 727–733.

6 Holshue ML, DeBolt C, Lindquist S, et al. First case of 2019 novel coronavirus in the United States. *N Engl J Med* 2020; 382: 929–936.

7 Chu DK, Leung CY, Gilbert M, et al. Avian coronavirus in wild aquatic birds. *J Virol* 2011; 85: 12815–12820.

8 Lewandowski K, Xu Y, Pullan ST, et al. Metagenomic nanopore sequencing of influenza virus direct from clinical respiratory samples. *J Clin Microbiol* 2019; 58: e00963–19.

9 Kafetzopoulou LE, Efthymiadis K, Lewandowski K, et al. Assessment of metagenomic Nanopore and Illumina sequencing for recovering whole genome sequences of chikungunya and dengue viruses directly from clinical samples. *Euro Surveill* 2018; 23: 1800228.

10 Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* 2020; 395: 565–574.

11 World Health Organization. Coronavirus disease 2019 (COVID-19). Situation report 53. 13 Mar 2020. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200313-sitrep-53-covid-19.pdf?sfvrsn=adb3f72_2 (viewed 14 Mar 2020).

12 Phan LT, Nguyen TV, Luong QC, et al. Importation and human-to-human transmission of a novel coronavirus in Vietnam. *N Engl J Med* 2020; 382: 872–874.

13 Australian Department of Health. Coronavirus (COVID-19) health alert. https://www.health.gov.au/news/health-alerts/novel-coronavirus-2019-ncov-health-alert#travellers-and-visitors (viewed 13 Mar 2020). ■

Supporting Information

Additional Supporting Information is included with the online version of this article.

FORM 43A

Rule 43.06(3)

IN THE SUPREME COURT OF VICTORIA AT MELBOURNE
COMMON LAW DIVISON
JUDICIAL REVIEW AND APPEALS LIST

No. S ECI 2021/03031

B E T W E E N

JONATHAN EDWARD KINGSFORD ANDREWS — Plaintiff

-and-

PROFESSOR BRETT SUTTON
(in his capacity as Chief Health Officer
as designated under the *COVID Omnibus (Emergency Measures) Act 2020*) — First Defendant

ASSOCIATE PROFESSOR MICHELLE GILES
(in her capacity as Deputy Health Commander
as authorised to exercise emergency powers by
Chief Health Officer under section 199(2)9(a) of
the *Public Health and Wellbeing Act 2008*) — Second Defendant

CERTIFICATE IDENTIFYING EXHIBIT

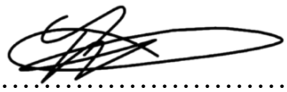
Date of Document:	24 August 2021	Solicitors Code:	141108
Filed on behalf of:	Jonathan E. K. Andrews	DX:	
Prepared by:	Jonathan E. K. Andrews	Telephone:	(03) 9070 1286
	Bosanquet Solicitors,	Ref:	210801
	TOK Corporate Centre, Level 1	Email:	info@bosanquets.com
	459 Toorak Rd, TOORAK VIC 3142		

This is the exhibit marked "**JA-07**" now produced and shown to JONATHAN EDWARD KINGSFORD ANDREWS at the time of swearing that person's affidavit on 24 AUGUST 2021:

……

JONATHAN EDWARD KINGSFORD ANDREWS

The Affidavit & Annexures were sworn to and signed by the Deponent by audio visual link and I, as affidavit taker, have used a scanned or electronic copy of the affidavit & annexures and not the original, in completing the jurat requirements under subsection s 27 ss (1) of the Oaths and Affirmations Act 2018 (VIC).



……

Mr Tony Nikolic, Director

Ashley, Francina, Leonard & Associates

Level 25, Tower 3, 300 Barangaroo Avenue,

Sydney, NSW, 2000

(An Australian Legal Practitioner as per Legal Profession Uniform Law [VIC])

Exhibit "JA-07"

Australian Department of Health, Freedom of Information Request.

FOI review rights

If you are dissatisfied with my decision, you may apply for a review.

Internal review

Under section 54 of the Act, you may apply in writing to the department for an internal review of my decision. The internal review application must be made within 30 days of the date of this notice (or such further period as the department allows). To assist in the internal review process, please provide reasons you consider review of my decision is necessary. The internal review will be undertaken by another officer of the department within 30 days of receipt of your application.

An application for an internal review can be sent to:

Email: FOI@health.gov.au
Mail: FOI Unit (MDP 516)
Department of Health
GPO Box 9848
CANBERRA ACT 2601

Information Commissioner review

Alternatively, under section 54L of the Act, you may apply to the Office of the Australian Information Commissioner (OAIC) for review of my decision. An application for review must be made in writing within 60 days of this notice (if you do not request an internal review).

You may also make a complaint to the OAIC about action taken by the department in relation to your application.

The OAIC can be contacted by:

Email: enquiries@oaic.gov.au
Phone: 1300 363 992

More information about OAIC review and making a complaint is available on the OAIC website:
https://www.oaic.gov.au/freedom-of-information/reviews/

Relevant provisions of the Act

The Act, including the provisions referred to in this letter, can be accessed from the Federal Register of Legislation website:
https://www.legislation.gov.au/Details/C2020C00366

Additional information

While the department does not hold the documents you have requested, I can provide the following information outside the Act, which I trust will be of assistance to you.

The Medical Journal of Australia (MJA) has published a paper on the isolation of SARS-CoV-2 at VIDRL. This paper describes the process of the inoculation of Vero/hSLAM cells from a nasopharyngeal swab, which led to the isolation of the SARS-CoV-2 virus in culture. The MJA paper is available here: https://www.mja.com.au/journal/2020/212/10/isolation-and-rapid-sharing-2019-novel-coronavirus-sars-cov-2-first-patient

The Communicable Diseases Network Australia (CDNA) has provided information on the guidelines used by public health units to respond to diseases and assists in providing nationally consistent advice across Australia. More information about the CDNA and the guidelines are available on the department's website at: https://www1.health.gov.au/internet/main/publishing.nsf/Content/cdna-song-novel-coronavirus.htm

I trust this information is of assistance to you

Contacts

If you require clarification of any of the matters discussed in this letter you should contact the department's Freedom of Information Unit on (02) 6289 1666 or at FOI@health.gov.au.

Yours sincerely

K Bishop
Principal Lawyer
Legal Advice & Legislation Branch

10 December 2020

FORM 43A

Rule 43.06(3)

IN THE SUPREME COURT OF VICTORIA AT MELBOURNE
COMMON LAW DIVISON
JUDICIAL REVIEW AND APPEALS LIST

No. S ECI 2021/03031

B E T W E E N

JONATHAN EDWARD KINGSFORD ANDREWS — Plaintiff

-and-

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(in his capacity as Chief Health Officer
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ASSOCIATE PROFESSOR MICHELLE GILES
(in her capacity as Deputy Health Commander
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Chief Health Officer under section 199(2)9(a) of
the *Public Health and Wellbeing Act 2008*) — Second Defendant

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Filed on behalf of:	Jonathan E. K. Andrews	DX:	
Prepared by:	Jonathan E. K. Andrews	Telephone:	(03) 9070 1286
	Bosanquet Solicitors,	Ref:	210801
	TOK Corporate Centre, Level 1	Email:	info@bosanquets.com
	459 Toorak Rd, TOORAK VIC 3142		

This is the exhibit marked "**JA-08**" now produced and shown to JONATHAN EDWARD KINGSFORD ANDREWS at the time of swearing that person's affidavit on 24 AUGUST 2021:

……

JONATHAN EDWARD KINGSFORD ANDREWS

The Affidavit & Annexures were sworn to and signed by the Deponent by audio visual link and I, as affidavit taker, have used a scanned or electronic copy of the affidavit & annexures and not the original, in completing the jurat requirements under subsection s 27 ss (1) of the Oaths and Affirmations Act 2018 (VIC).

……

Mr Tony Nikolic, Director

Ashley, Francina, Leonard & Associates

Level 25, Tower 3, 300 Barangaroo Avenue,

Sydney, NSW, 2000

(An Australian Legal Practitioner as per Legal Profession Uniform Law [VIC])

Exhibit “JA-08”

CSIRO Freedom of Information Request.

LEGAL
www.csiro.au

GPO Box 1700 Canberra ACT 2601
Telephone (02) 6276 6431 • **ABN** 41 687 119 230
Email: foi@csiro.au

7 October 2020

Our ref: FOI 2020/50

[redacted]

FREEDOM OF INFORMATION REQUEST – DECISION FOI2020/50

I refer to your request of 7 September 2020, under which you sought access under the *Freedom of Information Act 1982* (FOI Act) to:

> *"All records in the possession, custody or control of CSIRO describing the isolation of a SARS-COV-2 virus, directly from a sample taken from a diseased patient, where the patient sample was not first combined with any other source of genetic material (i.e. monkey kidney cells aka vero cells; lung cells from a lung cancer patient).*
>
> *Please note that I am using "isolation" in the every-day sense of the word: the act of separating a thing(s) from everything else. I am not requesting records where "isolation of SARS-COV-2" refers instead to:*
>
> - *the culturing of something, or*
> - *the performance of an amplification test (i.e. a PCR test), or*
> - *the sequencing of something.*
>
> *Please also note that my request is not limited to records that were authored by CSIRO or that pertain to work done by CSIRO. My request includes any sort of record, for example (but not limited to) any published peer-reviewed study that CSIRO has downloaded or printed.*
>
> *If any records match the above description of requested records and are currently available to the public elsewhere, please provide enough information about each record so that I may identify and access each record with certainty (i.e. title, author(s), date, journal, where the public may access it)."*

Decision maker

I am an authorised decision maker under section 23 of the FOI Act. This letter sets out my decision and reasons for the decision in relation to your request.

Decision

CSIRO has been unable to identify any document relevant to your request. I must therefore refuse access, pursuant to section 24A of the FOI Act on the basis that the document[s] sought do not exist or cannot be found.

Searches conducted

Searches were conducted by The Australian Centre for Disease Preparedness (formerly the Australian Animal Health Laboratory) and relevant staff in CSIRO's Business Units, and it was confirmed that CSIRO does not hold any documents relevant to the scope of your request.

Rights of Review

In accordance with section 26(1)(c) of the FOI Act, a statement setting out your rights of review under the Act is at Attachment A. Since my decision is that no documents exist, an application for review would be limited to a situation where you consider that I have not identified all the documents in the CSIRO's possession that are relevant to your request.

Yours sincerely,

Beth Maloney
Senior Legal Counsel
CSIRO

Attachment A

Review rights

You are entitled to seek review of this decision.

Internal Review

Firstly, under section 54 of the FOI Act, you may apply for an internal review of the decision. Your application must be made by whichever date is the later between:

30 days of you receiving this notice; or 15 days of you receiving the documents to which you have been granted access.

An internal review will be conducted by a different officer from the original decision-maker. No particular form is required to apply for review although it will assist your case to set out in the application the grounds on which you believe that the original decision should be overturned. An application for a review of the decision should be addressed to:

FOI Coordinator,
FOI@csiro.au

If you choose to seek an internal review, you will subsequently have a right to apply to the Australian Information Commissioner for a review of the internal review decision.

External review by the Australian Information Commissioner

Alternatively, under 54L of the FOI Act, you may seek review of this decision by the Australian Information Commissioner without first going to internal review. Your application must be made within 60 days of you receiving this notice.

The Information Commissioner is an independent office holder who may review decisions of agencies and Ministers under the FOI Act. More information is available on the Information Commissioner's website www.oaic.gov.au.

You can contact the Information Commissioner to request a review of a decision online or by writing to the Information Commissioner at:

GPO Box 2999
Canberra ACT 2601

Complaints to Ombudsman or Information Commissioner

You may complain to either the Commonwealth Ombudsman or the Information Commissioner about action taken by CSIRO in relation to the application. The Ombudsman will consult with the Information Commissioner before investigating a complaint about the handling of an FOI request.

Your enquiries to the Ombudsman can be directed to:

Phone 1300 362 072 (local call charge)
Email ombudsman@ombudsman.gov.au

Your enquiries to the Information Commissioner can be directed to:
Phone 1300 363 992 (local call charge)
Email enquiries@oaic.gov.au

There is no particular form required to make a complaint to the Ombudsman or the Information Commissioner. The request should be in writing and should set out the grounds on which it is considered that the action taken in relation to the request should be investigated and identify CSIRO as the relevant agency.

FORM 43A

Rule 43.06(3)

IN THE SUPREME COURT OF VICTORIA AT MELBOURNE
COMMON LAW DIVISON
JUDICIAL REVIEW AND APPEALS LIST

No. S ECI 2021/03031

B E T W E E N

JONATHAN EDWARD KINGSFORD ANDREWS — Plaintiff

-and-

PROFESSOR BRETT SUTTON
(in his capacity as Chief Health Officer
as designated under the *COVID Omnibus (Emergency Measures) Act 2020*) — First Defendant

ASSOCIATE PROFESSOR MICHELLE GILES
(in her capacity as Deputy Health Commander
as authorised to exercise emergency powers by
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CERTIFICATE IDENTIFYING EXHIBIT

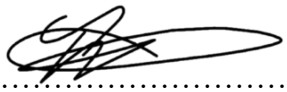
Date of Document:	24 August 2021	Solicitors Code:	141108
Filed on behalf of:	Jonathan E. K. Andrews	DX:	
Prepared by:	Jonathan E. K. Andrews	Telephone:	(03) 9070 1286
	Bosanquet Solicitors,	Ref:	210801
	TOK Corporate Centre, Level 1	Email:	info@bosanquets.com
	459 Toorak Rd, TOORAK VIC 3142		

This is the exhibit marked "**JA-09**" now produced and shown to JONATHAN EDWARD KINGSFORD ANDREWS at the time of swearing that person's affidavit on 24 AUGUST 2021:

……

JONATHAN EDWARD KINGSFORD ANDREWS

The Affidavit & Annexures were sworn to and signed by the Deponent by audio visual link and I, as affidavit taker, have used a scanned or electronic copy of the affidavit & annexures and not the original, in completing the jurat requirements under subsection s 27 ss (1) of the Oaths and Affirmations Act 2018 (VIC).



……

Mr Tony Nikolic, Director

Ashley, Francina, Leonard & Associates

Level 25, Tower 3, 300 Barangaroo Avenue,

Sydney, NSW, 2000

(An Australian Legal Practitioner as per Legal Profession Uniform Law [VIC])

Exhibit “JA-09”

Bibliography of materials relied upon.

BIBLIOGRAPHY OF MATERIAL RELIED UPON

1. *Emergency Powers, Public Health and COVID-19 Research Paper No. 2, August 2020,*Mclean and Huf, Department of Parliamentary Services Parliament of Victoria.

2. *External peer review of the RTPCR test to detect SARS-CoV-2 reveals 10 major scientific flaws atthe molecular and methodological level: consequences for false positive results* (McSheehy,
Angelova, Franchi et al) 30 November 2020, 10.5281/zenodo 4298004.

3. *Predicting Infectious SARS Cov-2 from Diagnostic Samples, Clinical Infectious Diseases, 71(10),2663-2666* (Bullard, J & Dust, K & Funk et al).

3. *Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR* (Corman, Drosten et al),Eurosurveill. 2020;25(3):pii=2000045. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045.

4. *WHO Information Notice for IVD Users 2020/05, Nucleic acid testing (NAT) technologies that use polymerase chain reaction (PCR) for detection of SARS-Cov-2, 20 January 2021*, World Health Organisation.

5. *Extracellular Vesicles Derived From Apoptotic Cells: An Essential Link Between Death and Regeneration*, Maojiao Li1 et al, Frontiers in Cell and Developmental Biology, 2020 October https://www.frontiersin.org/articles/10.3389/fcell.2020.573511/full, Website accessed 13 August
2021.

6. Dr AndreZ Kaufman, µStatement of Virus Isolation (SOVI)¶, Website accessed 13 August 2021,https://andrewkaufmanmd.com/sovi.

7. *COVID-19 vaccine: Pfizer Australia - COMIRNATY BNT162b2 (mRNA) - approved for use inindividuals 12 years and older*, Australian Government Health Department, COVID-19 Vaccines, 23 July 2021, Website accessed 18 August 2021 https://www.tga.gov.au/covid-19-vaccine-pfizer-australia-comirnaty-bnt162b2-mrna-approved-use-individuals-12-years-and-older.

8. *Is it true? Were COVID-19 vaccines rushed through approvals or given emergency use authorisations in Australia?,* Department of Health, Website accessed 18 August 2021,
https://www.health.gov.au/initiatives-and-programs/covid-19-vaccines/is-it-true/is-it-true-were-covid-
19-vaccines-rushed-through-approvals-or-given-emergency-use-authorisations-in-australia.

9. *COVID-19 indemnity scheme to protect health professionals and patient, Ministers Department of*
Health, published 2 July 2021, Website accessed 18 August 2021, *COVID-19 indemnity scheme to protect health professionals and patient, Ministers Department of Health*, published 2 July 2021, Website accessed 18 August 2021, https://www.health.gov.au/ministers/the-hon-greg-hunt-mp/media/covid-19-indemnity-scheme-to-protect-health-professionals-and-patients.

10. *Assessment and Testing Criteria for coronavirus (COVID-19)*, Victoria Department of Health,Website accessed 18 August 2021, https://www.dhhs.vic.gov.au/assessment-and-testing-criteria- coronavirus-covid-19.

11. Expert Opinionative Report by Prof. Dr. rer. biol. hum. Ulrike Kämmerer
Molecular biologist, RT-PCR specialist and Cell biologist, trained Virologist (Diploma, PhD- thesis) and Immunologist (Habilitation).

CASE LAW

1. *Loielo -v- Giles* [2020] VSC 722.

2. *Borrowdale -v- Director General of Health* [2020] NZHC [290] Thomas, Venning and Ellis JJ.

3. *Tribunal da Relacao de Lisboa 3a Seccao Proc. No.* 1783/20.7T8PDL.L1, 11 November 2020 (Portugal).

4. *FPÖ Wien -v- Landespolizeidirektion Wien* VGW-103 / 048 / 3227 / 2021-2 (Austria).

STATUTORY LAW

1. *Charter of Human Rights and Responsibilities Act 2006* ss 7(2), 12, 21, 38, 39.

2. Public Health and Wellbeing Act 2008 ss 4, 5, 6, 7, 8, 9, 10, 11, 111, 197, 198, 199, 200.

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ASSOCIATE PROFESSOR MICHELLE GILES — Second Defendant
(in her capacity as Deputy Health Commander as authorised to exercise emergency powers by Chief Health Officer under section 199(2)9(a) of the *Public Health and Wellbeing Act 2008*)

CERTIFICATE IDENTIFYING EXHIBIT

Date of Document:	24 August 2021	Solicitors Code:	141108
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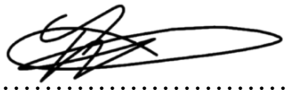
This is the exhibit marked "**JA-10**" now produced and shown to JONATHAN EDWARD KINGSFORD ANDREWS at the time of swearing that person's affidavit on 24 AUGUST 2021:

……

JONATHAN EDWARD KINGSFORD ANDREWS

The Affidavit & Annexures were sworn to and signed by the Deponent by audio visual link and I, as affidavit taker, have used a scanned or electronic copy of the affidavit & annexures and not the original, in completing the jurat requirements under subsection s 27 ss (1) of the Oaths and Affirmations Act 2018 (VIC).



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(An Australian Legal Practitioner as per Legal Profession Uniform Law [VIC])

Exhibit “JA-10”

Expert Opinionative Report by Prof. Dr. rer. biol. hum. Ulrike Kämmerer.

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Expert opinion

by Prof. Dr. rer. biol. hum. Ulrike Kämmerer

Molecular biologist, RT-PCR specialist and Cell biologist, trained Virologist (Diploma, PhD-thesis) and Immunologist (Habilitation).

Address: Dpt. OB/Gyn, Research Lab, University Hospital of Würzburg, Josef-Schneider-Str. 4, D-97080 Würzburg, Germany.

Regarding the evidentiary question, "What is the power of the RT-qPCR assay and currently used rapid tests to detect SARS-CoV-2 coronavirus infection?"

1. Nucleic acid detection by RT-qPCR test

Prefix: Reverse **t**ranscriptase-**q**uantitative **p**olymerase **c**hain **r**eaction (RT-qPCR) assays are unsuitable as a diagnostic tool for the detection of an active infection with SARS-CoV-2 for numerous reasons.

1.1 Explanation of terms/basics

In a **polymerase chain reaction (PCR)**, a defined short piece of deoxyribonucleic acid (DNA) (usually 100-1000 bases) is amplified using the enzyme polymerase. The piece of DNA to be amplified is flanced with the help of two very short single-stranded DNA segments, the "primers".

These **primers** usually consist of a defined sequence of 18-25 nucleic acid bases (the primer sequence) that specifically match the regions on the DNA that flank the section to be amplified. To ensure PCR specificity, these primers must explicitly match only this flanking region and no other region of a DNA. With the help of large gene databases and corresponding software programs (e.g. primer blast https://www.ncbi.nlm.nih.gov/tools/primer-blast/), these primers can be selected highly specifically in the PCR design. Specialized companies then synthesize the molecular chains from the submitted primer sequences and deliver them to the PCR laboratory or the manufacturer of PCR kits. Here, these primers must then be tested with valid positive and negative controls under a wide variety of experimental conditions and optimized in use. This ensures that only the DNA searched for is detected and amplified with the primer pair used, and that no other similar DNA segments are detected.

Once the primers have been found and are specific, the DNA to be amplified can be mixed with the primer pair, various auxiliary chemicals and the polymerase enzyme in a reaction batch and the chain reaction started.

PCR procedure: The following steps are repeated in cycles.

1. the mixture is boiled at over 90°C (denatured). This separates the DNA strands, which are usually present as a double strand, into single strands to enable the subsequent attachment of the primers.
2. During the subsequent cooling down to the so-called "**annealing temperature**", the primers can attach to their matching regions on the separated DNA strands. The binding of the primers, the annealing, only occurs in a narrowly limited temperature range, the so-called melting temperature. This depends mainly on the base composition of the primers and therefore their sequence will ideally always be chosen so that both primers have the same melting temperature of about 60°C. The annealed primers form the DNA strands. The attached primers form the starting point for the polymerase.
3. starting from the primers, this polymerase completes the single-stranded DNA, which is present due to the heating, into a matching double strand (**elongation**), usually at approx. 72°C.

Due to the position of the two primers on the flanking sides of the sought DNA section, the elongation reactions on the single strands are in opposite directions, since the polymerase always works in one direction only. At the end of this step, two identical new double-stranded DNA molecules have now been created from an original double-stranded DNA, which are separated again by boiling, then amplified into 4 identical DNA molecules with the aid of primer addition and the polymerase, and so on.

Each PCR cycle consisting of boiling-annealing-elongation causes a doubling of the DNA section sought, so that the amplification takes place in the logarithm of 2 and thus an extremely high number of copies of the original starting material is available very quickly.

Thus, after 10 PCR cycles, 210 = 1,024 DNA copies are obtained from one DNA strand, after 20 cycles already more than 1 million (1,048,576) and after 30 cycles more than 1 billion (1,073,741,824) copies

In the **quantitative PCR (qPCR)** technique, as currently used worldwide mainly for the detection of genomic RNA from SARS-CoV-2, a third short piece of DNA, similar to the two primers, is used which can bind appropriately in the middle of the DNA section sought, the "**probe**". Unlike the two primers (which are nucleic acids only), this probe is additionally bound to two molecules, a fluorescent dye at one end and another molecule (quencher), which can prevent the emission of fluorescence as long as both are simultaneously (i.e. in close proximity to each other) on the sample. During the elongation step, the polymerase now degrades this probe. This separates the quencher from the fluorescence molecule and the latter can now emit its color signal. This color signal is detected and measured in the device performing the PCR (thermocycler). Thus, with each PCR cycle, more and more fluorescence signals are released according to the increasing number of copies, the probe "glows" more and more.

And the curve of color signal intensity increases with each cycle. At a certain value, the curve then exceeds the background noise (threshold) and is considered positive. The number of cycles at which this threshold is exceeded is referred to as the **CT value** (CT stands for "cycle threshold").

The faster the fluorescence rises (lower CT), the more initial copies of the DNA sought were present in the PCR approach. Since neither the primers nor the enzyme polymerase always work 100% specifically, a fraction of non-specific DNA is also copied in each PCR run. And the more cycles the PCR runs, the greater the risk that even these few non-specific reactions will exceed the threshold value. Therefore, from a CT value of 40, a false positive signal due to non-specific starting materials must be assumed with the greatest probability. A reliable PCR should therefore require no more than 30-35 cycles to generate a clear "positive" signal; in the case of active infections with sought-after viruses, a sufficient number of cycles of 25-30 can be assumed (see also point 3.2.).

The **reverse transcriptase reaction (RT)** is required if the starting nucleic acid to be amplified is not present as DNA but as **ribonucleic acid (RNA),** as is the case with SARS-CoV-2 as an RNA virus. Since only DNA can be amplified in PCR, an RNA must first be converted into DNA. This is done with the help of the enzyme "reverse transcriptase", which creates a complementary copying strand of DNA from RNA, which then serves as the starting material for the PCR.

In order to evaluate the reliability of a result obtained by RT-qPCR or even PCR, the sensitivity and specificity of the test system used are evaluated using defined samples of diluted correct target genes (e.g. RNA of the sought virus) and very similar, but not sought target genes (e.g. closely related viruses).

The **sensitivity** indicates how sensitively the test can detect even the smallest amounts of the target gene sought, while the **specificity** describes how reliably the test excludes the possibility that other, closely related genes can also lead to a positive result (**false positive**). The higher the specificity, the more certain it is that the PCR system itself will not produce false positive results.

However, this does not exclude false positive events, which can be caused by laboratory contamination with target genes, contamination of test chemicals and contamination directly during sample collection. These contamination-related false positive results can be excluded by rigorous quality assurance and standard operating procedures (SOPs), the use of specially trained personnel and permanent external control in the form of interlaboratory comparisons.

1.2 Basic information on diagnostic significance

The inventor of the PCR test, Nobel Prize winner Kary Mullis, who died in August 2019, repeatedly pointed out that his test is solely suitable for making a molecule (deoxyribonucleic

acid, DNA) or fragment of DNA, which is otherwise invisible to the human eye, visible by amplification. But not to allow a statement on whether what has been made visible is dangerous or causes illness.

In particular, a PCR test - even if performed correctly - cannot provide any information on whether a person is infected with an active pathogen or not. This is because the test cannot distinguish between "dead" matter*, such as a completely harmless genome fragment as a remnant of the body's own immune system's fight against a cold or flu (such genome fragments can still be found many months after the immune system has "taken care" of the problem), and "living" matter, i.e. a "fresh" virus capable of reproducing. Explicitly, this is listed as a disadvantage of PCR on the information sheet of the Swiss Federal Office for Civil Protection FOCP Spiez Laboratory as follows: "*Only pathogens whose gene sequence is known can be detected*. ***Whether a pathogen is infectious (virulent, "alive") or not remains unknown***" (https://www.labor-spiez.ch//pdf/de/dok/pos/88_021_Plakate_PCR_d.pdf) . Also Marion Koopmanns, the director of the Department of Viral Sciences at Erasmus University and expert advisor to WHO, thus one of the central virologists of the Corona question and at the same time co-author on the RT-qPCR publication of Corman/Drosten in Eurosurveillance confirms in an interview with NPO Radio 1 (26.11. 2020 as part of her podcast "Virusfeiten" https://www.nporadio1.nl/podcasts/virusfeiten/46542/4-blijvend-moe-na-corona-misschien-helpt-een-aspirientje) in response to the presenter's statement that the PCR test does not necessarily show that one is contagious, that this is correct, because the PCR shows whether one has viral RNA on one's person (minute 0:09 in https://www.youtube.com/watch?v=flsF7trvq2c)

Explicitly the decisive passage of the interview with Marion Koopmanns (MK):

"MK: ...there are some stories circulating that say, well, the PCR test is not good.

Interviewer: At least it doesn't necessarily show that you're contagious.

MK: Yeah, exactly. And that's also true. Because the PCR shows that you have the viral RNA with you. That's literally what PCR does. And whether that RNA is in a virus particle that is still intact and also infectious. Or whether it's just residual RNA that can be detected long after infection. There is no way to distinguish between the two. You can get a feel for it by looking up "How much is there?". But you can't tell that difference very well. That is: this test is great for saying "you've had it", but this test is less good for saying "at this point you're still infectious".

Interviewer: you're talking about the PCR test right now, aren't you?

MK: Yes."

In original: (Dutch)

" MK: ...er ciruleren wat verhalen waarin gezegd wordt, nou ja, de PCR test ist niet goed. Interviewer: Althans die toont niet persee aan dat je besmettelijk bent.

MK: Ja precies. En dat klopt ook. Want de PCR toont aan dat jij het virus RNA bij je hebt. Dat is letterlijk wat de PCR doet. En of dat RNA in een virus deeltje zit dat nog intact is en ook besmettelijk is. Of dat

het gewoon restjes RNA zijn, die je nog een tijd lang nadat iemand geinfecteerd is geweest, kunt aantonen, dat onderscheid zie je niet. Je kunt een beetje een gevoel krijgen door te kijken "hoeveel is het?". Maar dat verschil is niet goed te maken. Dat betekent, die test is prima om te zeggen "je hebt het gehad", maar die test is minder geschikt om te zeggen "op dit moment ben je nog besmettelijk".

Interviewer: Over de PCR test heb je het nu, huh?

MK: ja“

Translated with www.DeepL.com/Translator (free version)

* For example, PCR is also used in forensics to amplify residual DNA present from hair remains or other trace materials by means of PCR in such a way that the genetic origin of the perpetrator(s) can be identified ("genetic fingerprint").

The Swedish Ministry of Health states on its official website (https://www.folkhalsomyndigheten.se/publicerat-material/publikationsarkiv/v/vagledning-om-kriterier-for-bedomning-av-smittfrihet-vid-covid-19/): "*The PCR technology used in tests to detect viruses cannot distinguish between viruses capable of infecting cells and viruses that have been rendered harmless by the immune system**, and therefore these tests cannot be used to determine whether someone is infectious or not.** RNA from viruses can often be detected for weeks (sometimes months) after infection but does not mean that a person is still infectious*." In the original note: "*PCR-tekniken som används i test för att påvisa virus kan inte skilja på virus med förmåga att infektera celler och virus som oskadliggjorts av immunförsvaret och därför kan man inte använda dessa test för att avgöra om någon är smittsam eller inte. RNA från virus kan ofta påvisas i veckor (ibland månader) efter insjuknandet men innebär inte att man fortfarande är smittsam.*" This assessment was confirmed on 19.04.2021.

So, even if everything is done "correctly" when performing the PCR including all preparatory steps (PCR design and establishment, sample collection, preparation and PCR performance) and the test is positive, i.e.: detects a genome sequence, which may also exist in one or even the specific "Corona" virus (SARS-CoV-2), this does not mean under any circumstances that the person, who was tested positive, must be infected with a replicating SARS-CoV-2 and thus infectious = dangerous for other persons.

Rather, for the detection of an active infection with SARS-CoV-2, further, and specifically diagnostic methods such as the isolation of reproducible viruses must be used (gold standard).

1.3 Factors influencing the reliability of the PCR test

In fact, however, the results of a PCR test depend on a number of parameters which, on the one hand, cause considerable uncertainties and, on the other hand, can be specifically manipulated in such a way that many or few (apparently) positive results are obtained.

1.3.1 Number of independent target genes ("targets")

The protocol "Diagnostic detection of Wuhan coronavirus 2019 by real-time PCR" (https://www.who.int/docs/default-source/coronaviruse/wuhan-virus-assay-v1991527e5122341d99287a1b17c111902.pdf), originally published by WHO on 13/01/2020, described the sequence of PCR detections of **three independent partial genes** of the virus later renamed SARS-CoV-2. The sequence referred to the E gene, the RdRp gene, and then the N gene. Already on 17.01.2020 a change followed by the WHO with the protocol "Diagnostic detection of 2019-nCoV by real time PCR" (https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c_2) in which the N-gene was removed as detection and thus instead of the original three targets only **two targets** were recommended. On March 02, 2020, a WHO test protocol "Laboratory testing for coronaviurs disease 2019 (COVID-19) in suspected human cases" (https://apps.who.int/iris/bitstream/handle/10665/331329/WHO-COVID-19-laboratory-2020.4-eng.pdf?sequence=1&isAllowed=y), which was updated again, pointed out that *„.... In areas where COVID-19 virus is widely spread a simpler algorithm might be adopted in which for example screening by RT-PCR of **a single discriminatory target** is considered sufficient.....*" (page 3 below), whereupon the laboratories widely switched to analyzing only one target. As a result, many laboratories specialized only in the E gene detection as the sole target as a valid PCR, as e.g. explicitly described by the Augsburg laboratory on 03.04. (only still available in the Internet cache: https://www.oder-spree-piraten.de/wp-content/uploads/2020/05/Ge%C3%A4ndertes-Befundlayout-der-SARS-CoV2-PCR-Ergebnisse-_-Labor-Augsburg-MVZ-GmbH.pdf

However, the outstanding importance of the number of independent target genes analyzed by PCR results from the following calculation:

The three targets E, RdRp and N gene originally specified in the WHO protocol for the detection of SARS-CoV-2 were rapidly used in many laboratory and commercial test systems. An interlaboratory comparison by Institut Instant e.V. (https://corona-ausschuss.de/wp-content/uploads/2020/07/Instand-Ringversuch-Virusgenom-Nachweis-SARS-CoV-2.pdf) showed a mean specificity for these genes of:

Target of the SARS-CoV-2 genome	Number of test analyzed	**Specificity A** (test sample cell culture without virus)	**Specificity B** Test Sample contains related Coronavirus (HCoV 229E)	Mean % detected correct	Mean specificity absolute	Mean error rate (1-abs. Spec.)

E-gen	24	99,46%	95,17%	97,31	0,9731	0,0269
RdRp-gen	13	97,80%	90,66 %	94,23	0,9423	0,0577
N-gen	21	98,20%	87,95 %	93,08	0,9308	0,0692

In a mixed population of 100,000 tests, even no true infected person would result due to the mean error rate:

For E-only genetic test: 100,000 x 0.0269 = 2690 false positives.

For E and RdRp test in sequence: 100,000 x (0.0269 x 0.0577) = 155 false positive

For all three genes (E, RdRp, N): 100,000 x (0.0269 x 0.0577 x 0.0692) = 10 false positives

This means that the specification of the WHO to successively reduce the number of target genes of SARS-CoV-2 to be tested from three to one resulted in an increase in the number of persons tested falsely positive in the above calculation example from 10 with three genes to almost 3000 with only the E gene per 100,000 tests performed. If the 100,000 tests carried out were representative of 100,000 citizens of a city/county within 7 days, this question of the target genes used alone would result in a difference of 10 compared to 155 compared to 2690 with regard to the "/-daily incidence" and, depending on this, the severity of the restrictions on the freedom of the citizens taken.

Evaluation: The calculation example also shows how daily case numbers can be manipulated by "playing to the specifications" regarding the targets to be detected for the laboratories. In view of the immense impact on political decisions, which are determined by the absolute numbers of positive tests and the "7-day incidence" derived from them, the specification of the WHO (and also of the RKI) to reduce the target genes has clearly been suitable to artificially inflate the "pandemic" by a factor of 300 by wrong test specifications.

This is an evidence-free procedure, which on the one hand entails enormous personal restrictions of quarantine/isolation, which the falsely "positively tested" persons have to suffer, and on the other hand willingly accepts the enormous social and economic restrictions and damages via the "7-day incidence number".

If the correct target number of three or even better (as e.g. in Thailand) up to six genes had been consistently used for PCR analysis, the rate of positive tests and thus the "7-day incidence" would have been reduced almost completely to zero.

1.3.2 Number of cycles performed (CT value)

In addition to the number of target genes detected, especially in the case of only one or a maximum of two genes, the number of cycles of amplification in the qPCR up to the "positive" result and the resulting CT value are decisive factors. **The smaller the CT value of a sample in a qPCR, the higher the initial amount of DNA in the sample.** Under standardized conditions,

this correlates with (in the case of viruses) the initial amount of viruses, the so-called **viral load,** which should ideally be expressed as "number of viral copies" per ml of sample. This viral load also correlates in the case of SARS-CoV-2 with the cultivability of infectious viruses in cell culture as published with the participation of C. Drosten already in March 2020. (Figure 1e in Wölfel et al., https://doi.org/10.1038/s41586-020-2196-x)

Here, a minimum quantity of 10^6 RNA copies/ml was necessary in order to be able to grow viruses from the sample, in another work from the group of C. Drosten (from May 2021) even an average of 10^8 viruses in the sample was necessary for a positive cell culture (supplemental Figure S4 from https://pubmed.ncbi.nlm.nih.gov/34035154/). In the latter work, it was also found that none of the 25,381 individuals tested had viral genomes per ml in the sample in the case of a determined viral load below 10^5 (Table S1), whereas RT-qPCR from the original protocol (Corman V et al., 10.2807/1560-7917.ES.2020.25.3.2000045) can already deliver a positive result at approx. 4 copies per sample preparation (5µl corresponding to approx. 10^3 copies/ml), i.e. already by a factor of 1000-10000 earlier than in a sample with an actual infectious virus load.

Even **commercial PCR test systems**, so-called “kits”, sometimes show detection limits of less than 10 copies/reaction, such as kits from the company TIB-Molbiol (https://www.roche-as.es/lm_pdf/MDx_53-0777_96_Wuhan-R-gene_V200204_09155376001%20%282%29.pdf).

In technical terms, a distinction must be made here between "colonization" of the throat with a few individual viruses that do not cause infection and a genuine "infection". The latter is accompanied by viruses capable of multiplying, which then leads to a) a symptomatic illness and b) infectivity, i.e. the ability to infect other persons.

Christian Drosten already described this aspect in 2014 in an interview in the “Wirtschaftswoche” (https://www.wiwo.de/technologie/forschung/virologe-drosten-im-gespraech-2014-die-who-kann-nur-empfehlungen-aussprechen/9903228-2.html) in connection with MERS: "*Yes, but the method* (note: PCR is meant) *is so sensitive that it can detect a single hereditary molecule of this virus. If such a pathogen, for example, flits across a nurse's nasal mucosa for just one day* (note: this would be the above-mentioned "colonization") *without her becoming ill or otherwise noticing anything about it, then suddenly she is a Mers case. Where previously deathly ill people were reported, now suddenly mild cases and people who are actually perfectly healthy are included in the reporting statistics.*" [....] "*Because what is initially of interest are the real cases* (Note: These are the "infected"). ***Whether asymptomatic or mildly infected hospital workers are really virus carriers, I think, is questionable. Even more questionable is whether they can pass the virus on to others***." The latter is a crucial statement also with respect to the SARS-CoV-2 viruses, which are very closely related to MERS. But it is precisely this point about virus transmission (and thus driving the pandemic) that is the rationale for the intervening measures such as quarantine/isolation orders, the "lockdowns," and the so-called AHA rules.

Further evidence for the relevance of the CT value

A **Canadian study** by Jared Bullard/Guillaume Poliquin in Clinical Infectious Deseases 2020, which can be read at the link (https://doi.org/10.1093/cid/ciaa638), came to the conclusion as early as May 2020, that **no reproducible virus was found above a CT value of 24.** This means that the attempt to subsequently cultivate reproducible viruses from smear samples that only resulted in a positive test at a higher CT value failed. According to this study, above a CT value of 24, the amount of detectable viral genetic material is so low that the positive test could no longer be interpreted in terms of an active infection. A large study by Jaffar et al. (10.1093/cid/ciaa1491) set the limit for the cultivability of SARS-CoV-2 from patient sample material at a CT value of 30.

In a study comparing antigen testing/RT-qPCR and virus cultivation from the CDC (https://academic.oup.com/cid/advance-article/doi/10.1093/cid/ciab303/6224406) , successful virus cultivation was described for a CT range of 17.4-28.8, where only at a CT of <25 all specimens were from symptomatic individuals and were associated with successful virus cultivation. When the CT was between 25 and 29, in 18.2% Virus isolation was positive. In the original: *"Virus was isolated from specimens with Ct values ranging from 17.4-29.8; virus was isolated from all specimens with a Ct value <25 and from 18.5% (5/27) of specimens with a Ct value ≥25.*" (center of page 9). Irrespective of this check using virus cultivation, however, all samples that were positive in two target sequences from the "N gene" with a CT up to 40 were considered "true positive".

In his NDR podcast of Feb. 16, 2021, C. Drosten explicitly named that an increase in CT from 25-27 across the border of 28 means that individuals from whom these smears were obtained with the higher CT are no longer infectious. "*And again, you see a Ct shift from 25 to 27 approximately, 27, 28. And that's a range where, in our estimation, that's really where infectivity ends. If you see such a patient sample and you would ask, is the patient still infectious, I would say: No, this is now slowly no longer an infectious area. You can correlate that*." page 4 (top right column in: https://www.ndr.de/nachrichten/info/coronaskript270.pdf)

With these CT data, C. Drosten presumably refers mainly to a study on vaccine efficacy in Israel, which was verified by RT-qPCR. This study, which was available in a preprint publication ("Decreased SARS-CoV-2 viral load following vaccination" (https://www.medrxiv.org/content/10.1101/2021.02.06.21251283v1) and in the meantime is available regularly under the title " Initial report of decreased SARS-CoV-2 viral load after inoculation with the BNT162b2 vaccine (https://www.nature.com/articles/s41591-021-01316-7") is also referred to in a letter from the German Robert Koch Institute (RKI; AZ: ID3176 of 31.03.2021) to the German Federal Ministry of Health. In this study, PCR tests after vaccination (with BNT162b2) show that in vaccinated subjects who become positive for SARS-CoV-2 in the PCR from day 12 after the first vaccination, the CT for the three tested genes (E, N, RdRp using

the Seegene Allplex test kit, which has a specificity of 96-98.4 according to the Instant EQA scheme 340) increases from a mean CT of 25 to a mean CT of 27.

Compared with a similarly SARS-CoV-2 PCR positive unvaccinated cohort, this study establishes vaccination success based on a CT decrease of 1.64-2.33. *„Finally, applied on all infections (post-vaccination and unvaccinated, n=5,794), a multivariate linear regression model accounting for age, sex and vaccination quantify* ***Ct regression coefficients ranging from 1.64 (N gene) to 2.33 (RdRp) for vaccination after 12 days*** *or longer prior to infection sampling*", which is arithmetically equivalent to a 4-fold reduction in viral load in vaccinated versus unvaccinated. In the original: : *„As a difference of 1 Ct unit is equivalent to a factor of about 1.94 in viral particles per sample, these Ct differences represent a viral load ratio ranging from 2.96 to 4.68.*"

It is also noteworthy in the PCR analyses description, that the CT values up to 40 were analyzed and evaluated in this work (Extended Data Figure 4).

Accordingly, in a new recommendation dated April 16, 2021, the CDC also addresses the CT in SARS-CoV-2 PCR to the effect that it should have a value of no more than 28 in order to send PCR products from "vaccine breakthroughs" (i.e., RT-qPCR positive individuals after complete vaccination) to the laboratory for sequencing (https://www.cdc.gov/vaccines/covid-19/downloads/Information-for-laboratories-COVID-vaccine-breakthrough-case-investigation.pdf).

Also, a study from South Korea mentions a **CT of ≤ 25** as the upper limit of clinically relevant "positives" and uses this value for comparison with the goodness of antigen tests. Original quote: " [....] based on a clinically significant Ct value of ≤ 25 (....)" (p. 3 in https://jkms.org/DOIx.php?id=10.3346/jkms.2021.36.e101).

Unanimous scientific opinion (including Dr. Fauci of the US CDC, but also a number of scientists quoted in the New York Times in August 2020, https://www.nytimes.com/2020/08/29/health/coronavirus-testing.html) is **that all "positive" results detected only after a cycle of 35 have no scientific (i.e.: no evidence-based) basis**. In contrast, the RT-qPCR test for the detection of SARS-CoV-2, propagated worldwide with the help of the WHO, was (and following it all other tests based on it as a blueprint) set to 45 cycles without defining a CT value for "positive".

Also as early as May 2020, a position paper was issued by the National Centre for Infectious Disease in Singapore (https://www.ncid.sg/Documents/Period%20of%20Infectivity%20Position%20Statementv2.pdf), which points out that.

1. it is important to note that viral RNA detection by PCR does not equate to infectiousness or viable virus

2. the cycle threshold value (CT) of the PCR, as a surrogate marker for the viral RNA content, already detects viral RNA from a CT of 30, but no longer the presence of replicable viruses and the persons concerned are not infectious.

Original text extract "*6. A surrogate marker of 'viral load' with PCR is the cycle threshold value (Ct). A low Ct value indicates a high viral RNA amount, and vice versa. As noted above,* ***detection of viral RNA does not necessarily mean the presence of infectious or viable virus****. In a local study from a multicenter cohort of 73 COVID-19 patients, when the Ct value was 30 or higher (i.e. when viral load is low), no viable virus (based on being able to culture the virus) has been found.*"

The RKI also states on its homepage as of 11.08.2020. (https://www.rki.de/DE/Content/InfAZ/N/Neuartiges_Coronavirus/Vorl_Testung_nCoV.html#doc13490982bodyText4) "*First results from diagnostics at RKI show that loss of cultivability in cell culture was associated with an RNA amount of <250 copies/5 µL RNA determined by real-time PCR (note: is RT-qPCR). This RNA level corresponded to a Ct value >30 in the test system used*."

A recent study from South Korea (https://www.nejm.org/doi/full/10.1056/NEJMc2027040) **sets the limit for virus cultivability at a CT value of 28.4.**

In another recent study from Frankfurt (https://www.mdpi.com/2077-0383/10/2/328), it was shown that of 64 RT-qPCR positive patient samples (one gene tested), virus cultivation in cell culture was only possible from 33 (=52%). These infectious samples were already positive up to a mean CT value of 26 (Supplementary Figure 1), whereas virus cultivation was no longer possible from the samples with a higher CT.

In the round robin Instant e.V. (http://www.finddx.org/covid-19/pipeline/?section=molecular-assays#diag_tab.) see also next point, the enormous range of CT values even for highly standardized samples between the different laboratories and also with regard to the different target genes becomes apparent. For example, here the CT for the same defined diluted sample of SARS-CoV-2 (sample number 340061) for the WHO-recommended genes varies between 15-40 (E gene), 20-40.7 (N gene) and 19.5-42.8 (RdRp gene). **This impressively demonstrates an extreme lack of test standardization within the participating (and certified) laboratories.**

Against this background, it is disconcerting when RT-qPCR is still considered the "gold standard" by the RKI (and WHO) without defining the exact validations and external certification conditions (and without these apparently being fully monitored by the authorities).

Assessment:

In general, RT-qPCR cannot detect intact, propagable (infectious) viruses, not even the complete intact viral genome, but only nucleic acid of the sought section. In principle, it is possible to define a threshold (CT), above which a positive PCR signal no longer correlates with replication viruses, by validation with a parallel virus cultivation in cell culture for well adjusted and correctly performed PCR tests. This has been a well-practiced routine in blood product monitoring (for HIV and Hepatitis viruses) for years.

This stringent validation then allows - as long as the test system is NOT changed - as a surrogate marker an estimation of the viral load and thus the possible infectivity of the tested sample, but never definitive detection. As soon as a component of the PCR test system (be it chemicals, plastic goods, enzymes, protocol procedures or machines) is changed in one of the applied steps, it is mandatory to recalibrate the system.

From all the information published so far (see above), it can be assumed that any CT value above 35 is no longer associated with the cultivability of infectious viruses and is therefore the absolute threshold for the decision "positive", also irrespective of the test system used. The CT range 25-35 may still be validly assessed as "positive in the sense of infectivity" in a test-dependent manner if, as described, it has been compared with a virus cultivation by adequate validation in the performing laboratory.

CT≤ 25 : positive

CT 26-35 : positive only if matched with viral culture

CT > 35 : negative

The strict evaluation of the CT value mainly matters when the target number is one, but generally applies to each individual target.

The threshold CT 25 was already introduced in December 2020 by the English "Office of national statistics (ONS), here with CT above 25 as negative. Table sheet 2 (Data) in the linked Excel data sheet (link below). Results: " *„The analysis shows: - People with a higher concentration of viral genetic material (positive cases with low Ct values; below 25) are more likely to be infectious in a household than those with lower concentrations (positive cases with high Ct values; above 25).”* (https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/conditionsanddiseases/adhocs/12683coronaviruscovid19infectionsurveycyclethresholdandhouseholdtransmissionanalysis).

With reference to this ONS threshold, the authors of a large cohort study from Münster, Germany (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8166461/), using RT-qPCR of the

ORF-1ab and E genes in smear samples from 162457 individuals, concluded that : „*RT-PCR test positivity should not be taken as an accurate measure of infectious SARS-CoV-2 incidence*“.

This study showed that a total of 2.6% of the samples had a positive RT-qPCR result. The CT threshold above which the samples were considered definitely negative was set at 40. The samples were also analyzed according to the number of samples that became positive up to a cutoff value of 25 (always both genes, personal information on request to A. Spelsberg).

The results showed that in asymptomatic individuals only 0.4% (68 of 16,874 individuals) had a positive RT-qPCR test with a mean CT of almost 29. Of these, only 27% (= 18 individuals) had a CT of up to 25, which was considered by the authors “*indicating a likelihood of the person being infectious*“. Converted, this means that in only 18 of 16874 (=0.1%) asymptomatic (healthy) individuals did PCR indicate possible infectivity with respect to SARS-CoV-2.

Also, of 6212 symptomatic individuals from the peak periods of the first two "Corona waves," only 403 individuals (=6.5%) had a positive RT-qPCR for SARS-CoV-2 with a mean CT of 27.8 (1st wave) and 26.6 (2nd wave). Of these positives, a maximum (in the 2nd wave) of 40% (= 145/367) and in the first wave even only 26.5% (=10/36) individuals had a CT of up to 25 and could thus be classified as probably infectious. Consequently, only 155 of 6212 symptomatic (ill) persons (=2.5%) could be assumed to be possibly infectious with SARS-CoV-2.

Values from Table 1 of: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8166461/

In this respect, it can be assumed that all CT values higher than 30 may no longer be used to assess whether the person from whom the sample was obtained "is infectious", but rather the UK ONS CT of 25 must be used.

However, taken on its own, without information about the matching with the specific number of viral genomes (viral load) and the correlation with a cultivability of corresponding virus quantities, the CT value even at low numbers is worthless as an evaluation criterion of a positive PCR detection.

1.3.3 Adequate controls

In order to **correctly assess sensitivity and specificity of an RT-qPCR**, adequate samples must be included in each reaction run. This begins at the test site with "**blank swabs**" to reliably exclude contamination at the sample collection site, continues with **extraction controls** to ensure the correct isolation of reproducible RNA with all subsequent processing steps, i.e. an artificially produced defined RNA which is carried and processed in all steps of sample preparation up to PCR and for which PCR is then also carried out with the aid of suitable

primers. This excludes the possibility of inhibitory substances or errors preventing the amplification of RNA during sample processing.

Such defined controls have been available through Instant e.V. since November 2020. From the booklet accompanying the shipment of the defined (https://www.instand-ev.de/fileadmin/uploads/user_upload/Dokumente/Virologie/20210118g_Begleitheft_-_quantitative_Bezugsproben_1_und_2_-_SARS-CoV-2.pdf), the following aspects can generally be identified:

- The strain BetaCoV/Munich/ChVir984/2020 was used as a control as a heat-inactivated sample with controlled viral counts corresponding to 10^6 and 10^7 RNA copies/ml, since this was the threshold for assessing patients as "probably contagious" (in: 2.2. Intended use). According to the database (https://www.european-virus-archive.com/virus/human-2019-ncov-isolate), this strain was obtained in Munich on 28.01.2020 and is sold via Charité.

- Depending on the tested genes and the performing laboratory, a wide range of CT values was shown despite defined sample in reference laboratories. For example, this CT value varied for the E gene for the sample with 106 copies/ml between 21.9 (lab 4) and 28.7 (lab 1) and the RdRp gene between 24.8 (lab 4) and 33.0 (lab 1) with the N gene already at a CT of 22.1. Across all tests from reporting laboratories, there was a spread of CT values of 12-38 for this sample (Figure 2) and a spread of CT values of 10-36 for the higher concentration sample. This example alone shows that each laboratory must always carry the defined samples in each test series in order to be able to convert the laboratory's own CT value to the viral load, which should be the actual reference sample for assessing the tested patient sample.

The extreme variation of CT values in the different test systems is addressed by C. Drosten in his NDR Podcast 94 of June 22, 2021 (https://www.ndr.de/nachrichten/info/coronaskript306.pdf) as follows: "*And namely the Ct values that we have here, they are not easily comparable between the individual test manufacturers."...." But we can only compare them numerically as long as we are in the same test system. The differences there are sometimes considerable. There are test manufacturers for whom a value of, say, 25 is nothing at all worrying, while the same value of 25 in another manufacturer's test shows that this is already a seriously infectious concentration. That's simply because these test manufacturers don't standardize on the Ct value*." (pg. 17) He further complains that this standardization does not take place with calibrations (produced by him) (pg.18): "*What does not happen at the moment, however, is that nationwide recommendations are also made and applied by the state health offices or also by the Robert Koch Institute for certain areas of application on this technical laboratory basis that has now been created*." Means: since autumn 2020, suitable controls for virus load determination would be available and would have to be requested by the authorities for the laboratories to validate the test results, but this is obviously not happening (according to C. Drosten). "***We***

can even do it in such a way that this inherent problem of non-comparability of Ct values is already solved. Mind you, in the fall. The technique and the lab testing is not the catch here, but it's again the implementation and the regulation."

Furthermore, each correct test series must include **a series of external negative controls** (i.e., carried in parallel as patient samples) and a positive control, ideally consisting of an inactivated defined SARS-CoV-2 virus strain. This would be an original task of the RKI an other official government organizations like the CDC or WHO (with the assistance of other suitable public institutions such as the Bernhard Nocht Institute or the Friedrich-Löffler Institute in Germany) to isolate a sufficient number of SARS-CoV-2 viruses from patient samples in the laboratory facilities available there (safety level 4). Those institutions then could cultivate defined strains from these as controls, to inactivate these and to deliver them in defined virus numbers as controls to the testing laboratories via the local supervisory authorities. However, since this important service is still not offered even after more than a year of the "pandemic", the positive control usually consists of a synthetic RNA that only encodes the target genes of the test system. This positive control can also be used to determine the lower detection limit of the PCR. This is specified by some commercial kits as 20 or fewer viral genomes per sample and thus (see point 1.3.2.) already detects a virus quantity in the smear that is below the infectious dose by a factor of 10^5, i.e. has no diagnostic/prognostic value whatsoever. An overview of the currently used commercial kits with their line data can be found at http://www.finddx.org/covid-19/pipeline/?section=molecular-assays#diag_tab.

Interlaboratory tests:

Correctly performed controls also include the participation of the laboratories performing the tests in so-called "interlaboratory comparisons" (see also 1.3.1.). In these, an anonymized panel of test samples is made available by an external provider. In the case of virus detection, these contain negative samples and samples with closely related viruses (inactivated) to check the specificity (these samples must not give a positive signal) and positive samples with different dilutions of the virus sought (inactivated) to determine the sensitivity (from which number of viruses does the PCR become positive, with which CT value).

In the case of SARS-CoV-2, the first EQA scheme "Virus Genome Detection - SARS-CoV-2 (340)" by the association "INSTANT e.V." was ready in April 2020. According to the report, 488 laboratories participated in this EQA scheme, of which 463 reported results. The results can be read in the published commentary (Zeichhardt M: “Kommentar zum Extra Ringversuch Gruppe 340 Virusgenom-Nachweis SARS-CoV-2", available at: https://corona-ausschuss.de/wp-content/uploads/2020/07/Instand-Ringversuch-Virusgenom-Nachweis-SARS-CoV-2.pdf) and show two deviations from the usual EQA procedure, which already here pointed to laboratory problems with RT-qPCR for the detection of SARS-CoV-2:

For example, page four of the publication states, "*Important evaluation notice: only 4 of the 7 samples tested in this Extra EQAS will be considered for obtaining a certificate of successful participation*." The footnote on page 10 of the commentary states, "*In the April 17, 2020 interim evaluation, all participants in the Extra INSTAND EQA trial (340) Virus Genome Detection of SARS-CoV-2 April 2020 were notified ahead of time of the sample characteristics of samples 340059, 340060, and 340064. The results of these 3 samples will not be considered for the granting of a certificate* [....]" The reason for this exclusion of certain samples is explained on page 4 of the commentary: "*While the extra ring trial was still running, INSTAND e.V. received urgent requests from Germany and abroad to reveal the properties of the samples to be tested before the end of the extended submission period, i.e., before April 28, 2020, so that laboratories can improve their test method in the short term in case of possible incorrect measurements*." (page 4 above in INSTANT e.V. report))

This procedure is very unusual for a real round robin test and thus no longer represents an independent external verification procedure of the participating laboratories.

Despite the samples already detected and the reduced test scope, sample mix-ups occurred in a large number of laboratories - as stated on page 18 of the commentary: "*For sample 340064 (SARS-CoV-2 positive diluted 1 : 100 000), the reduced success rate of only 93.2 % is essentially based on incorrect result assignments (mix-ups) for sample 340064 and sample 340065 (negative for SARS-CoV-2 and positive for HCoV 229E). The mix-ups for samples 340064 and 340065 involved 24 laboratories with a total of 59 results per sample. See also section 2.4.2.1* [...]." Thus, a large number of laboratories mistakenly confused sample 340064 (slightly diluted SARS-CoV-2) with sample 340065 (negative for SARS-CoV-2 and positive for the closely related virus HCoV 229E).

Apart from the startling fact that a considerable number of samples were obviously mixed up even under highly standardized procedures in an interlaboratory comparison (which raises the question of the corresponding rate of sample mix-ups and thus wrongly assigned swab samples under mass testing conditions), it is striking that all reported mix-ups concerned only these two samples, but not the samples with the final numbers 61 (very highly diluted SARS-CoV-2) and 62 (negative), which were also evaluated. The detailed results of a second round robin test from June/July 2020 (https://www.instand-ev.de/System/rv-files/Zusammenfassung%20der%20Probeneigenschaften%20und%20Sollwerte%20Virologie%20340%20Juni%20Juli%202020%2020200911a.pdf) are still not publicly available.

1.3.4 Exclusion of contaminations of reagents and "problems in the course of action".

The best PCR design can still lead to false positive results if either the underlying reagents / kits are contaminated with positive samples or, much more likely, contamination occurs in the laboratory workflow. Since PCR is an extremely sensitive method (exponential reaction

course) that can detect few molecules of a DNA, laboratory contamination by PCR end products is a major problem in clinical diagnostics (described e.g. already in 2004 in Aslanuadeh J et al., http://www.annclinlabsci.org/content/34/4/389.full.pdf+html: *„A typical PCR generates as many as 10^9 copies of target sequence and if aerosolized, even the smallest aerosol will contain as many as 10^6 amplification products [6]. If uncontrolled, within a relatively short time the buildup of aerosolized amplification products will contaminate laboratory reagents, equipment, and ventilation systems [6]*.)

This extreme risk of contamination requires that the diagnostic laboratories working with PCR take the utmost care in testing - very competent staff, contamination-proof environment, permanent independent control.

Already in the above mentioned round robin 340 in April a problem with false positive results appeared, which was commented as follows (page 20 below): "*In addition, in some cases the tests with the SARS-CoV-2 negative control samples 340060, 340062 and 340065 indicate specificity problems, which are independent of mix-ups of the samples 340064 and 340065. Clarification is needed as to whether these false positives are due to a specificity problem with the tests used or to carryover of SARS-CoV-2 during test performance or to mix-ups with other samples in this EQA at the laboratories in question*." (page 21 bottom in https://www.instand-ev.de/System/rv-files/340%20DE%20SARS-CoV-2%20Genom%20April%202020%2020200502j.pdf). For mix-up in this EQA scheme, see details point 3.3. end of paragraph.

If, against this background, one further sees how, for example, according to a BBC report, work is carried out openly and extremely contamination-prone with untrained personnel in large test laboratories in England (https://www.youtube.com/watch?v=Uk1VK1reNtE), it is not surprising if even in Germany and other countries (where such reports have not yet been filmed) occasional reports of "false positive cases" due to laboratory contamination are found in the media (e.g. MVZ Augsburg - link at the end of the section). Even under controlled laboratory conditions, contamination due to PCR steps cannot be safely excluded in such a highly sensitive method. Thus, the problem of false positive PCR results in SARS-CoV-2 diagnostics due to laboratory procedures and already pointed out in the first publication of RT-qPCR (Corman et al., 10.2807/1560-7917.ES.2020.25.3.2000045): *„In four individual test reactions, weak initial reactivity was seen but they were negative upon retesting with the same assay"* [.....] *„.... most probably to handling issues...."*

Even if the course of action in the laboratory functions optimally and is extremely monitored in order to greatly minimize laboratory-related contamination, an unexpected source of false positive results can arise here in the **contamination of the materials/chemicals used ex-manufacturer**. For example, the swab materials used to take samples may already be contaminated ex works - as in the case of the "Phantom of Heilbronn", in which the cotton swabs used to take DNA traces at the crime scenes were contaminated with the DNA of a packaging worker from the manufacturer's plant, thus hampering forensics with false traces

for years (https://www.faz.net/aktuell/gesellschaft/kriminalitaet/dna-ermittlungspanne-das-phantom-von-heilbronn-ist-widerlegt-1925411.html).

In the case of SARS-CoV-2 diagnostics, a contamination problem due to PCR primers containing positive controls ex works was also published in June 2020 (Wernike et al., DOI: 10.1111/tbed.13684). Here, it had been noticed that even pure water samples with several independent primer batches gave unambiguous positive SARS-CoV-2 detection in RT-qPCR: "*However, there were also primers/sample sets that displayed very low-level contaminations, which were detected only during thorough internal validation*."

Also, some false-positive results of SARS-CoV-2 RT-qPCR testing reported in the daily press in summer 2020 were attributed to material issues (e.g., https://www.br.de/nachrichten/bayern/probleme-in-augsburger-labor-bringen-falsche-testergebnisse,SEh5Qq4) and e.g. in the United States where 77 football players were tested positive for SARS-CoV-2 and then a second confirming test revealed that the results of the first tests were all false positive (https://www.nfl.com/news/all-77-false-positive-covid-19-tests-come-back-negative-upon-reruns)

Evaluation:

Even with ideal RT-qPCR design and good laboratory practice with adequate validation, problems in daily handling procedures as well as externally via samples already contaminated ex-factory can significantly influence the quality of results of RT-qPCR and lead to false positive results.

1.3.5 Commercial PCR test kits: Approval for diagnostics?

Very early on, commercial PCR test systems, the "PCR kits", were used in routine laboratories for diagnostics, although the majority of them were declared for "RUO" ("research use only").

The first, and therefore most concise test manufacturer, the Berlin (Germany) company TIB Molbiol, whose company owner (Olfert Landt) was already listed as author on the WHO protocol recommendations alongside Christian Drosten, deserves special mention. The kits, which are accordingly based on the WHO recommendations, are used via the company Roche on their large-scale automatic machines "Cobas" and should therefore make up a large percentage of the kits used for routine diagnostics of SARS-CoV-2 in Germany and around the world.

Exact figures cannot be determined, however, TIB Molbiol has already delivered more than 60 million of these tests worldwide in 2020 according to its own information (https://www.tib-molbiol.de/de/covid-19), although these are still declared as "***Not tested for use in diagnostic procedures***" (e.g. header in https://www.roche-as.es/lm_pdf/MDx_53-0777_96_Wuhan-R-gene_V200204_09155376001%20%282%29.pdf). The corresponding package inserts with the

protocol information and kit descriptions of the company TIB Molbiol were astonishingly according to metadata of the originally available PDFs (can be provided electronically) already on 15.01.2020 (!!!) completely with ROCHE SAP number are still available unchanged (albeit with metadata analysis 06.02.2020) parallel to other test kits, which now have an approval for in vitro diagnostics.

In the meantime (as of July 2021), there is a wide range of PCR detection systems (https://www.theglobalfund.org/media/9629/covid19_diagnosticproducts_list_en.pdf), many of which are also approved for in vitro diagnostics (IVD) of SARS-CoV-2. (e.g. here: https://www.genesig.com/assets/files/Path_COVID_19_CE_STED_IFU_Issue_500.pdf). In the description of these kits it reads under 1. „Intended use: "***Positive results are indicative of the presence of SARS-CoV-2 RNA***. *Positive results do not rule out co-infection with other bacteriaor other viruses. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions.* ***Positive and Negative results must be combined with clinical observations, patient history, and epidemiological information***"

1.4 Relationship between positive nucleic acid detection in RT-qPCR, disease and infectivity.

Only those actually infected can pass on the virus and carry the risk of disease, and thus should be used to determine the progression of an infection rate and wave of disease

"*PCR detection is the standard test for diagnosing viral infections such as SARS-CoV-2. The test detects individual pathogen genes but not intact pathogens*." And, "*There is a possibility that the test will be positive beyond the duration of infection because "viral debris" is still present in the nose or throat. Reliable proof of infectivity is only possible with elaborate tests that involve laboratory testing to determine whether the material from the swabs can kill living cells*." This was written by the German medical journal "Dt. Ärzteblatt" on 02/01/2021 (https://www.aerzteblatt.de/nachrichten/120745). Also, the CDC points out under "Disadvantages" of NAATs (nucleic acid amplification tests = PCR) "**A positive NAAT diagnostic test should not be repeated within 90 days, because people may continue to have detectable RNA after risk of transmission has passed**" (below in summary table at: https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antigen-tests-guidelines.html#previous)

"*The PCR assay detects gene segments of SARS-CoV-2; it does not tell us whether they are infectious viruses or viral remnants after passed through infection. This would require pathogen culturing*." Was stated in an August 2020 publication by the head of Frankfurt's public health department (https://www.laekh.de/fileadmin/user_upload/Heftarchiv/Einzelartikel/2020/10_2020/Die_Covid-19-Pandemie_in_Frankfurt_am_Main.pdf). And in his expert opinion of April 21 for a

court in Heidelberg (to be viewed anonymously here: https://www.corodok.de/wp-content/uploads/2021/05/Gutachten-Prof.-Drosten-v.-31.3.2021-anonymisiert.pdf), the expert C. Drosten confirms that an RT-PCR test can also be positive if "*at least the section to be detected from the genome of the virus is present in the tested sample*". This means that genetic material fragments can also yield positive results in the PCR without originating from an intact, replication competent virus, thus also providing an alleged virus detection in non-infectious samples.

In a CDC publication dated 7/13/20 titled "*CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel For Emergency Use Only Instructions for Use*", (https://www.fda.gov/media/134922/download), on p. 38 under the heading "*Limitations*" (still found on p. 37) : *"**• Detection of viral RNA may not indicate the presence of infectious virus or that 2019-nCoV is the causative agent for clinical symptoms."***

That a mRNA detection of SARS-CoV-2 does not necessarily correlate with disease and should not be used as the sole criterion for disease assessment, but is only an aid to confirm a clinical diagnosis, is also clearly described in WHO Information "*Notice for IVD Users 2020/05, Nucleic acid testing (NAT) technologies that use polymerase chain reaction (PCR) for detection of SARS-CoV-2*" dated Jan. 13, 2021 (published Jan. 20, 2021 at https://www.who.int/news/item/20-01-2021-who-information-notice-for-ivd-users-2020-05): **: „***Where test results do not correspond with the clinical presentation, a new specimen should be taken and retested using the same or different NAT technology.*"

Further: : "*Most PCR assays are indicated as an aid for diagnosis, therefore, health care providers must consider any result in combination with timing of sampling, specimen type, assay specifics, clinical observations, patient history, confirmed status of any contacts, and epidemiological information*"

Also in a recent publication in Lancet (https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(21)00425-6/fulltext#%20), the authors refer to the RT-qPCR assay as follows: : *„**In our view, current PCR testing is therefore not the appropriate gold standard for evaluating a SARS-CoV-2 public health test***", because, in their opinion, the PCR still comes up positive even when those tested are no longer positive, since the RNA can persist in the body for weeks and months even after the immune system has successfully combated it, without the person still being infectious. *„Once SARS-CoV-2 replication has been controlled by the immune system, RNA levels detectable by PCR on respiratory secretions fall to very low levels when individuals are much less likely to infect others. **The remaining RNA copies can take weeks, or occasionally months, to clear, during which time PCR remains positive**"*

In a May 2021 publication in Science led by C. Drosten (DOI: 10.1126/science.abi5273) examining the infectivity of SARS-CoV-2, the authors define in the very first sentence of the abstract the **parameters for quantifying and potentially passing on the virus as „... *viral load and whether samples yield a replicating virus isolate in cell culture*.**" They further state in the

introduction that viral load is determined by viral RNA concentration **and successful virus isolation in cell culture assays**. Furthermore, they point out that even "*.... viral load and cell culture infectivity cannot be translated directly to in vivo infectiousness, and the impact of social context and behavior on transmission is very high, these quantifiable parameters can generally be expected to be those most closely associated with transmission likelihood*".

In his NDR Podcast 94 of June 22, 2021 (https://www.ndr.de/nachrichten/info/coronaskript306.pdf) page 16, C. Drosten addresses the relationship between CT value and infectivity as follows: "*....that a case just because the **patient has a high CT value at this moment, that is, because he may not be infectious right now**, so he has little virus, he has virus, but he has little virus*,...."

1.5 Conclusion: Significance of RT-qPCR Tests for the Detection of SARS-CoV-2 Coronavirus Infection

1) In view of the problems outlined in section 1.3, RT-qPCR is not a suitable and reliable (and approved) diagnostic tool for the detection of infectious (replication capable) SARS-CoV-2 viruses.

Furthermore, the sole RT-qPCR test result is only a laboratory value, which, in view of the aspect outlined in point 1.4, does not permit any statement about the presence of infectious viruses and may only be used at all in conjunction with a clinical symptom diagnosis (ascertained by healthcare providers, in Germany medical doctors).

Summary: RT-qPCR is not suitable for the detection of SARS-CoV-2 infection in asymptomatic individuals by means of a nasopharyngeal swab, as is done uncritically in large numbers and predominantly by non-medical personnel WITHOUT (crucially here: contrary to the WHO requirement!) taking a medical history and ascertaining the symptoms of those tested.

2. Antigen detection by means of a rapid test

2.1 Explanation of terms/basics of the rapid test

The "rapid tests" currently used for the diagnosis of SARS-CoV-2 are based on the principle of an antigen test according to the "lateral flow" test procedure. This detects a protein component (protein) of the virus.

An **antigen** is a three-dimensional structure of proteins and other organic materials that can be recognized and bound by antibodies (immunoglobulins).

In the case of **viral antigens**, these are usually individual protein components (proteins) from the viral structure. These can be either complete structural proteins such as the "spike" protein located on the surface (S protein, these are the "stalked buttons" in the virus drawings) or the envelope protein ("envelope" - E protein) or that protein from which the nuclear envelope is built (nucleocapsid = N protein). Fragments of these complete structural proteins are also often sufficient to be bound by antibodies. These are the so-called **epitopes**, which also represent the actual antibody binding site on the intact structural protein. Each structural protein usually has a large number of epitopes, so that different antibodies can bind simultaneously to different epitopes of the same protein.

In case of SARS-CoV-2, the major antigens (the above-mentioned, S, E, and N proteins) are those that trigger an immune response in the body when infected with the virus. As a result, the body forms antibodies that specifically recognize these antigens, then bind to them (**antigen-antibody reaction**) to neutralize the viruses and render them destructible to immune cells.

This antigen-antibody reaction can be used in the laboratory to search for the antigens in any sample with synthetically produced antibodies.

The basic principle of the so-called **antigen tests** in the laboratory (these aim at the detection of antigens by antibodies, unlike RT-PCR, which detects nucleic acids) is that two matching antibodies are produced in vitro, which recognize two different epitopes of the antigen being searched for, a so-called "**antibody pair**". Both antibodies must be selected in such a way that they can only recognize and bind the desired epitope on the antigen sought, but not other structures on similar antigens. They must therefore be highly specific in order to be used in diagnostics. This **high specificity** of diagnostic antibodies is ensured in test development by matching them with many very similar epitopes. All antibodies that bind undesired epitopes are discarded until only one ideal antibody pair remains that meets the requirements of very high specificity, high binding property (sensitivity) and no mutual interference.

The antigen test is then built on this antibody pair, in which the antigen sought is bound by both antibodies simultaneously and is sandwiched between them like the fry inside the sandwich bun (hence "**sandwich test**").

For the lateral flow rapid antigen tests, which are currently used in broad-spectrum population testing for the detection of SARS-CoV-2 antigens, this sandwich test system is now used.

Here, the first of the two specific antibodies is bound to a carrier material in such a way that its antigen binding site points freely upwards. This is the later region in the rapid test where a color change gives the signal "positive". The second antibody is coupled with a detection system that is later responsible for the color reaction and is located as a depot directly next to the site in the rapid test at which the sample is dripped on.

Test procedure: If the antigen, in this case the protein of SARS-CoV-2, is present in the swab sample, it binds with the first specific antibody from the depot after dropping into the test field of the detection cassette. Capillary forces cause the mixture of antigen with bound first antibody and excess unbound antibody to migrate from the depot towards the test field. Here, the second specific antibody fixed there then binds the antigen with the first antibody already bound to it. The solution migrates beyond the test field over another field where the excess antibodies are captured (control field). The detection system of the test begins to show a **chemical color reaction** wherever the first antibodies are bound. In the control field, this is caused by the surplus first antibodies that are now bound here and have "brought along" the detection system, thus indicating that the test has in principle functioned without interference.

In the test field, there is only a color change if an antigen was actually in the sample and was bound via the second antibody fixed there. Since the antigen has already arrived at the test field with the first antibody and the detection system, the chemical color reaction also begins here, which leads to the color change (usually a violet stripe) at the test region.

Whenever the antigen sought is present in the swab sample, it can bind the first antibody and transport it together with the detection system to the fixed second antibody, which then intercepts this antigen-antibody-detection system complex and thus causes the positive signal at this point.

The color change at the test site (**signal "positive"**), which causes the visible stripes in the rapid test, is a chemical reaction and therefore can be influenced by reaction conditions such as pH or chemicals that come with the sample and is a clear weakness in the reliability of the test.

This explains the many videos circulating on the internet that detect SAR-CoV-2 using the rapid antigen tests in apple juice, red wine, beer, etc.

2.2 Basic information on the diagnostic significance of the rapid antigen test

Like RT-PCR, rapid antigen tests cannot in principle determine whether the viral antigen found belongs to an intact, infectious virus or is a remnant (fragment) of viruses that have been killed by the immune system.

Irrespective of this general limitation of the significance with regard to infectivity, rapid tests only have an indicative character, not a reliable diagnostic significance.

The most well-known rapid test before Corona times was the rapid pregnancy test, which works according to the same principle of the antibody-antigen test. However, here the pregnancy hormone (HCG) acts as an antigen. If this is present in sufficient quantity in the tested urine, the test indicates "positive" - in this case, presumably pregnant. However, the rapid test alone will never be sufficient as a well-founded proof of pregnancy; in this case, the doctor will use HCG detection in the blood as well as an ultrasound to make the diagnosis.

The rapid antigen tests for the detection of SARS-CoV-2 components can also only give an indication of possible colonization or infectivity and are subject to similar limitations as RT-qPCR.

2.3 Factors influencing the reliability of rapid antigen tests

2.3.1 Pre-test probability

In an infographic entitled "Understanding Corona rapid test results" (https://www.rki.de/DE/Content/InfAZ/N/Neuartiges_Coronavirus/Infografik_Antigentest_PDF.pdf?__blob=publicationFile), the RKI clearly explains how the probability that a test result is correct depends on the so-called **pre-test probability**, i.e., on the actual number of genuinely infected persons in the tested population. This aspect of pre-test probability applies to both the rapid antigen tests and equally to the RT-qPCR tests.

The calculation example presented by the RKI for the interpretation of the rapid antigen tests assumes a realistic scenario starting from a sensitivity (susceptibility) of the antigen tests of 80% and a specificity (reliability) of 98%, whereby it is also explicitly mentioned here (https://www.rki.de/DE/Content/InfAZ/N/Neuartiges_Coronavirus/Vorl_Testung_nCoV.html): "*The considerable differences in performance of the various commercially available tests must be taken into account here* (reference to: https://www.medrxiv.org/content/10.1101/2020.10.01.20203836v1)."

Assuming fife persons out of 10,000 tested are truly infected with SARS-CoV-2, 200 false positive tests and four true positive tests will still show up. This means that one truly infected

person per 10,000 would be missed, but 200 would get a false positive result and therefore have to be quarantined/isolated until RT-qPCR testing then gives the "all clear". This would mean in the case of a school test with e.g. 1000 students, that 20 would get a false "You are Corona positive" and the school would first be closed as an "outbreak site" until the retesting by RT-qPCR gives the all-clear. Such cases have already been reported in the press.

-For example, in Altdorf near Nuremberg (Germany), 29 of 180 high school students tested positive in a rapid antigen test; upon examination, 28 of them turned out to be negative (https://www.merkur.de/bayern/nuernberg/nuernberg-corona-bayern-test-fiasko-schnelltests-fehlerhaft-positiv-schule-altdorf-gymnasium-zr-90253265.html).

- In Potsdam (Germany), 12 of 36 teachers tested positive with a rapid antigen test and were sent into quarantine. After review, all test results turned out to be false positives (https://www.news4teachers.de/2021/03/sorgen-schnelltests-fuer-chaos-an-schulen-falscher-alarm-legt-grundschule-lahm/).

- Medscape even headlined, "*200 false positives, 8 detected, 2 missed - why pediatric and adolescent physicians are skeptical of mass rapid testing* (https://deutsch.medscape.com/artikelansicht/4909842)

And even if the rate of genuinely infected persons in the tested group were very high, as in the second calculation example from the RKI (with 1000 out of 10,000 tested persons), the hit rate of the rapid tests would be poor and 180 persons would receive a false positive result and 200 a false negative test. This is where the poor sensitivity of the test comes into play.

In the "*Hinweisen zur Bewertung der Ergebnisse aus AG-Testen*" (Note: Antigen rapid tests, *Notes on the evaluation of results from AG tests*) on the webpage of the RKI, the problem of false positive antigen tests is addressed: "*A positive test result by means of AG test triggers the suspicion of a transmission-relevant infection with the SARS-CoV-2 and requires a follow-up test by means of PCR to avoid false positive results. In view of the potentially significant consequences of incorrect results, there are high requirements not only for the sensitivity of antigen tests, but also for their specificity. Thus, with low prevalence/pretest probability and low test specificity, a high number of false-positive results and a corresponding additional burden on the ÖGD due to imposition and, if necessary, withdrawal of measures would have to be expected*." https://www.rki.de/DE/Content/InfAZ/N/Neuartiges_Coronavirus/Vorl_Testung_nCoV.html

2.3.2 **Sensitivity**

Due to the fact that in the antigen test there is no such strong (exponential) amplification of the output signal as in RT-qPCR, but only a limited signal amplification due to the chemical

color reaction, **this type of test is significantly less sensitive than the RNA detection by RT-qPCR used for comparison**.

This "underperformance" of rapid antigen tests is the subject of a Lancet article (https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(21)00425-6/fulltext#%20), but here the negative test result in the rapid antigen test (here called LTF, lateral flow test) is relativized to: *„[....] in all six observed cases, viral loads were very low (Ct ≥29 reflecting around <1000 RNA copies per mL in the laboratory used)—when LFT should be negative*."

A brand new study from Norway (https://pubmed.ncbi.nlm.nih.gov/33736946/) confirms this finding that in asymptomatic individuals, rapid tests have an unsatisfactorily high inaccuracy, and that only in symptomatic individuals are reasonably accurate detections made of those who are actually infected. The authors conclude: *„Our results indicate that the test correctly identified most infectious individuals. Nevertheless, the sensitivity is considerably lower than for PCR*"

In a comparison of rapid antigen tests (3 different manufacturers) with unspecified RT-qPCR results ("different RT-qPCR methods" in the original: "*using different RT-qPCR methods*") in 5066 cases, of which 101 (=2%) had a positive RT-qPCR result, the parallel antigen test had a sensitivity of only 42.6%, with 16 false positive results (0.32%) and 58 false negative (1.15%) results, compared to RT-qPCR, in which CT values up to 35 were considered positive (corresponding to a converted viral load of 31 RNA copies/ml).

Here, positive antigen tests correlated very well with a high viral load (mean $2.7x10^6$ copies/ml) and low CT (up to 22, Figure 4) and typical disease symptoms, with low viral load with less than 10^6 RNA copies/ml (note: considered to be the limit of infectivity, a.o. by the RKI) and especially asymptomatic individuals, the rapid tests were often negative, which is considered a deficiency in this publication (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8234263/pdf/main.pdf), but against the background of the identification of infectious individuals should be more reliable than RT-qPCR when evaluated with too high CT values.

Also C. Drosten explains in his podcast (No. 94 page 15) of22.06.2021 to the self-test (antigen test): "*There must be already proper virus to get the test positive. But there also has to be a good amount of virus to infect someone. This is a good match. Even in the thresholds, it's a good match. So we actually estimate that the infectivity threshold is about where the detection threshold of these antigen tests is.*" (https://www.ndr.de/nachrichten/info/coronaskript306.pdf)

This **alleged lack of sensitivity** is the most common criticism when the unreliability of rapid antigen tests is reported. For example, the Pharmaceutical Newspaper

(https://www.pharmazeutische-zeitung.de/in-der-praxis-deutlich-unzuverlaessiger-als-auf-dem-papier-123017/) writes: "*Rapid antigen tests could detect mostly "highly infectious people with high viral loads," Keppler explains. "However, it is not the case that an infection could be reliably excluded by the negative result of a rapid test*." Here, however, the basis is comparing the rapid antigen test with RT-qPCR and criticizing the fact that only some of the RT-qPCR positive swab samples also become positive in the rapid antigen test.

For example, in Epidemiological Bulletin 3/2021, the RKI reports on a study using rapid tests in a Stuttgart clinic (from page 11 in: https://www.rki.de/DE/Content/Infekt/EpidBull/Archiv/2021/Ausgaben/03_21.pdf;jsessionid=15E8B09E615AECED77C34439BB8052AF.internet051?__blob=publicationFile). Here, Table 1 shows that of 18 RT-qPCR positive for SARS-CoV-2 RNA asymptomatic individuals, only 7 also had a positive signal in the rapid antigen test, and of symptomatic individuals, 36 of 42. Accordingly, the discussion states, "*Because of the very limited sensitivity of the antigen test in asymptomatic individuals, single testing in this population cannot adequately exclude SARS-CoV-2 infection.* ***Highly contagious individuals with low Ct values*** **(i.e., high viral load)** ***are detected with adequate confidence*****.**" Here, the data show, "*At a Ct value of 22 or less, the detection rate of the antigen test was 100%*."

This example shows very clearly that a reliable antigen test, when performed correctly, correlates very well for symptomatic individuals with rapid response in RT-qPCR (low CT value), but not for asymptomatic, and only high CT value RT-qPCR positive, individuals. This speaks to the real-world significance of rapid antigen testing in terms of detecting a high viral load in symptomatic individuals. However, according to these data, the test is unsuitable for testing asymptomatic persons, both to reliably identify possibly infected persons and to reliably identify healthy persons as negative.

Such a finding was also obtained in the current Frankfurt study (https://www.mdpi.com/2077-0383/10/2/328), where three rapid antigen tests (there AG-RDT, antigen rapid diagnostic test) were matched with a viral culture from the same samples in cell culture and correlated to RT-qPCR. The authors write about this in the abstract: *„In contrast, three Ag-RDTs demonstrated a more significant correlation with cell culture infectivity (61.8–82.4%)“.* This means that from those samples that were positive in the antigen test, a positive result was also seen in the virus culture with a significantly higher hit rate than with the significantly more sensitive RT-qPCR "postives".

A recently published study by the CDC also points to the high concordance of the antigen test with actual interrogatable virus in a sample from symptomatic patients (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7821766/). Here, a commercial rapid antigen test was matched with a virus cultured in cell culture and RT-qPCR. It showed a high hit rate (positive result) of the antigen test only when the samples also contained replicating viruses. Here, viruses could be grown from 85 of the total 147 samples (=58%) which were positive in the rapid antigen test and RT-qPCR (here with a CT of approx. 22), but only from 11

of the 124 samples (= 9%) which were RT-qPCR positive (here with a CT of 33-34) but antigen rapid test negative.

Another study from the CDC (https://academic.oup.com/cid/advance-article/doi/10.1093/cid/ciab303/6224406) correlated a positive antigen test with a CT of less than 29, and the cultivability of virus in cell culture (also to CT29). All subject samples positive in RT-qPCR with a CT ≥35 were negative in antigen detection.

In a recent publication of the working group of Christian Drosten (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8026170/ he is the last author), in which different antigen tests are tested for reliability, the correlation "positive antigen test" and viral load as well as infectivity is explicitly described. Already in the abstract it says: *„The sensitivity range of most AgPOCTs overlaps with **SARS-CoV-2 viral loads typically observed in the first week of symptoms, which marks the infectious period in most patients**. The AgPOCTs with limit of detections that approximate virus concentrations at which patients are infectious might enable shortcuts in decision making in various areas of health care and public health*." A viral load of 2-9x10^6 copies per smear is specified as the detection limit, which would nevertheless only lead to success (= sufficiently high viral load) in 1/5 of cases when compared with virus cultivation in cell culture. *„In terms of analytical sensitivity, the detection range of most AgPOCTs was found to range between around 2 million and 9 million copies per swab (accounting for a systematic predilution), and thus corresponds to a concentration that can be expected to yield a virus isolation success rate of around 20% in cell culture*"

In general, it can be stated from these published data:

- Samples from which viruses can be grown in cell culture, i.e., which have a high (infectious) viral load, are identified with good accuracy by the rapid antigen tests and by RT-PCR with low CT (below 25), but in the vast majority originate from symptomatic individuals.

- Samples from which no viruses can be grown in cell culture are mostly negative in evaluated and correctly applied rapid antigen tests (except for false positives - see 2.3.3) and have high CT values (mostly above 33) in RT-qPCR. These samples are predominantly from asymptomatic tested individuals and prove that these random "positives" without clinical symptoms do not have an infectious viral load.

2.3.3 Reliability (**specificity**) - exclusion of false positive results

Many of the rapid antigen tests used have not yet undergone a regular conformity assessment procedure for CE marking and have so far only been granted special approval by the BfArM in accordance with §11 of the Medical Devices Act (https://www.bfarm.de/DE/Medizinprodukte/Antigentests/_node.html). In addition, these tests are widely performed by untrained, non-medical personnel or even as "self-tests".

Regarding this problem of performing rapid antigen tests, Professor Oliver Keppler, M.D., chief of virology at the Max Pettenkofer Institute at Munich's Ludwig Maximilian University, calls for the following in an article in the Jan. 13, 2021, issue of Pharmazeutische Zeitung (DOI: 10.1007/s00430-020-00698-8): "[....] *these tests would also absolutely have to be performed correctly. "This should be in the hands of trained professionals*," he says. "*Now there is the idea of recruiting large numbers of job seekers to perform such tests in nursing homes.* ***If untrained personnel are used, I'm concerned that the reliability of the test results will suffer even further***"

In a recent interview (https://www.br.de/nachrichten/wissen/virologe-keppler-kritisiert-corina-schnelltests-falsche-sicherheit,SUg0dZZ), O. Keppler comments on the question "*How reliable is a positive test result?"* as follows: "*Unfortunately, there are also problems with the specificity of the rapid antigen tests: Depending on the incidence and the test used, according to RKI data****, there are about ten "false" positives for every "true" positive****. This also has serious consequences for the person affected: Immediate report to the health department and quarantine until a negative PCR is obtained, compile contact lists. This causes a great deal of expense, loss of work and school for several days, and last but not least, unwarranted anxiety. It also further undermines confidence in the national testing strategy*."

A recent Cochran review article (https://www.cochrane.de/de/news/aktualisierter-cochrane-review-bewertet-zuverl%C3%A4ssigkeit-von-schnelltests-zum-nachweis-von-covid) also concludes that rapid antigen tests are significantly more reliable in symptomatic individuals than in asymptomatic tested individuals. However, even in symptomatic individuals, the reliability of the best of the rapid tests evaluated in this study is significantly limited, leading the authors to describe the following scenarios:

1. "In a population of 1000 symptomatic individuals, 50 of whom actually have COVID-19, these rapid tests can be expected to correctly identify approximately 40 individuals as COVID-19 infected and miss between 6 and 12 cases of COVID-19. Between 5 and 9 of the positive test results would turn out to be false positives upon review."

2. "In a group of 10,000 persons without symptoms, in which 50 persons are truly infected with SARS-CoV-2, between 24 and 35 persons would be correctly identified as virus carriers, and between 15 and 26 cases would be missed. One would have to expect that the tests would yield between 125 and 213 positive results and that between 90 and 189 of these positive results would actually be false positives.

Also in the current publication of the working group of C. Drosten at the Charité the problem of "false positives" - here however under controlled laboratory conditions - is pointed out and discussed. The tests reviewed reacted falsely positive with various common respiratory viruses -which was confirmed by negative RT-qPCR using E-gene detection. *"All negative samples that*

showed a SARS-CoV-2 false-positive result in AgPOCTs were retested and ***confirmed as false-positive*** *with SARS-CoV-2 RT-rtPCR.*" (page. 5 in https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8026170/ top.) The authors around C. Drosten consider a false positive rate of up to 3% as acceptable and in two tests even found exceptions of 5% false positives. *„We observed acceptable rates of false-positive results (<3%) with most AgPOCTs, but rates greater than 5% with two assays in particular"*

Thus, even under controlled laboratory conditions with skilled personnel, an average of 30 false positive results would be obtained with commercial rapid tests out of 1000 rapid tests due to cross-reactivity with other respiratory viruses or "unknown factors", according to the publication by C. Drosten. (*„thus, a specific factor other than the tested pathogens was likely to have caused positive signals*")

The fact that the high rate of false positive tests in large-scale testing in the population occurs at a time of low viral incidence is shown by an article in the German Ärztezeitung of 04.07.2021 (https://www.aerztezeitung.de/Wirtschaft/80-Prozent-der-positiven-Corona-Schnelltests-falsch-positiv-421053.html). At the end of the regular cold season (May), about 50% of rapid tests were already reported as false positive, and this rate increased until it reached 80% false positive tests in June. The data of the article are based on the information of the Hamburg senate on a small inquiry of the CSU parliamentary group. The evaluation was based on a total of 308,000 reported antigen rapid tests in Hamburg of which 218 tests were positive (= 0.07% of those tested) and after PCR confirmation then only 44 (=0.014% of all those tested) remain.

For the consequences of false positive results due to lack of test specificity, see under 2.3.1. "Pre-test probability

2.5 Conclusion:

The rapid antigen tests used for mass testing **cannot provide any information about infectivity**, since they can only detect protein components without any connection to an intact, replicable virus. 1.

1) In order to allow an estimation of the infectivity of the tested persons, the respective positive test (similar to RT-qPCR) would have to be individually compared with a cultivability of viruses from the test sample, which is impossible under the extremely variable and unverifiable test conditions.

2. The low specificity of the tests causes a high rate of false positives, which result in unnecessary personnel (quarantine) and societal (e.g. schools closed, "outbreak notifications") efforts until they turn out to be false alarms.

Statement

I assure that I have prepared the expert opinion impartially and to the best of my knowledge and belief and based on valuable and official scientific and public sources.

Ulrike Kämmerer